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(54) **Title:** COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

(57) **Abstract:** The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

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COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

STATEMENT OF GOVERNMENT SUPPORT

[01] This invention was made with United States Government support under CDC contract RO1 CI000099-01. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

[02] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

BACKGROUND OF THE INVENTION

[03] A problem in determining the cause of a natural infectious outbreak or a bioterrorist attack is the sheer variety of organisms that can cause human disease. There are over 1400 organisms infectious to humans; many of these have the potential to emerge suddenly in a natural epidemic or to be used in a malicious attack by bioterrorists (Taylor et al. Philos. Trans. R. Soc. London B. Biol. Sci., 2001, 356, 983-989). This number does not include numerous strain variants, bioengineered versions, or pathogens that infect plants or animals.

[04] Much of the new technology being developed for detection of biological weapons incorporates a polymerase chain reaction (PCR) step based upon the use of highly specific primers and probes designed to selectively detect certain pathogenic organisms. Although this approach is appropriate for the most obvious bioterrorist organisms, like smallpox and anthrax, experience has shown that it is very difficult to predict which of hundreds of possible pathogenic organisms might be employed in a terrorist attack. Likewise, naturally emerging human disease that has caused devastating consequence in public health has come from unexpected families of bacteria, viruses, fungi, or protozoa. Plants and animals also have their natural burden of infectious disease agents and there are equally important biosafety and security concerns for agriculture.

[05] A major conundrum in public health protection, biodefense, and agricultural safety and security is that these disciplines need to be able to rapidly identify and characterize infectious agents, while there is no existing technology with the breadth of function to meet this need. Currently used methods for identification of bacteria rely upon culturing the bacterium to effect isolation from other

organisms and to obtain sufficient quantities of nucleic acid followed by sequencing of the nucleic acid, both processes which are time and labor intensive.

[06] Mass spectrometry provides detailed information about the molecules being analyzed, including high mass accuracy. It is also a process that can be easily automated. DNA chips with specific probes can only determine the presence or absence of specifically anticipated organisms. Because there are hundreds of thousands of species of benign bacteria, some very similar in sequence to threat organisms, even arrays with 10,000 probes lack the breadth needed to identify a particular organism.

[07] The present invention provides oligonucleotide primers and compositions and kits containing the oligonucleotide primers, which define bacterial bioagent identifying amplicons and, upon amplification, produce corresponding amplification products whose molecular masses provide the means to identify bacteria, for example, at and below the species taxonomic level.

SUMMARY OF THE INVENTION

[08] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

[09] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456.

[10] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[11] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[12] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288.

[13] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

[14] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

[15] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 698.

[16] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1420.

[17] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 698 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1420.

[18] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 217.

[19] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167

[20] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 217 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167.

[21] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 399.

[22] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.

[23] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 399 and an

oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.

[24] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430.

[25] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1321.

[26] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1321.

[27] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 174.

[28] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 853.

[29] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 174 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 853.

[30] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 172.

[31] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1360.

[32] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 172 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1360.

[33] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[34] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.

[35] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681.

[36] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[37] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[38] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 315.

[39] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.

[40] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 315 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.

[41] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 346.

[42] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 955.

[43] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 346 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 955.

[44] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504.

[45] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1409.

[46] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1409.

[47] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323.

[48] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1068.

[49] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1068.

[50] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 479.

[51] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 938.

[52] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 479 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 938.

[53] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[54] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 315:1379, 346:955, 504:1409, 323:1068, 479:938.

[55] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583.

[56] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923.

[57] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923.

[58] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454.

[59] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1418.

[60] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1418.

[61] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 250.

[62] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.

[63] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 250 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.

[64] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 384.

[65] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 878.

[66] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 384 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 878.

[67] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694.

[68] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1215.

[69] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1215.

[70] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 194.

[71] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1173.

[72] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 194 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1173.

[73] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375.

[74] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.

[75] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.

[76] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 656.

[77] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1224.

[78] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 656 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1224.

[79] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618.

[80] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.

[81] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.

[82] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 302.

[83] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 852.

[84] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 302 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 852.

[85] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199.

[86] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.

[87] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.

[88] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 596.

[89] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1169.

[90] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 596 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1169.

[91] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150.

[92] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1242.

[93] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1242.

[94] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.

[95] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.

[96] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.

[97] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.

[98] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1168.

[99] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1168.

[100] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 454:1418, 250:902, 384:878, 694:1215, 194:1173, 375:890, 656:1224, 618:1157, 302:852, 199:889, 596:1169, 150:1242, 166:1069 and 166:1168.

[101] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437.

[102] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.

[103] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.

[104] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 530.

[105] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 891.

[106] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 530 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 891.

[107] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 474.

[108] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 869.

[109] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 474 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 869.

[110] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 268.

[111] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.

[112] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 268 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.

[113] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418.

[114] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.

[115] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.

[116] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318.

[117] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1300.

[118] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1300.

[119] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440.

[120] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.

[121] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.

[122] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219.

[123] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.

[124] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.

[125] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence

identity with SEQ ID NO: 1137 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 530:891, 474:869, 268:1284, 418:1301, 318:1300, 440:1076 and 219:1013.

[126] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325.

[127] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.

[128] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.

[129] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278.

[130] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.

[131] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.

[132] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 465.

[133] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1037.

[134] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 465 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1037.

[135] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 148.

[136] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1172.

[137] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 148 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1172.

[138] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 190.

[139] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.

[140] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 190 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.

[141] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266.

[142] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1094.

[143] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1094.

[144] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 508.

[145] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1297.

[146] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 508 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1297.

[147] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259.

[148] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.

[149] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.

[150] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 278:1039: 465:1037, 148:1172, 190:1254, 266:1094, 508:1297 and 259:1060.

[151] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376.

[152] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

[153] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

[154] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267.

[155] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.

[156] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.

[157] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705.

[158] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1056.

[159] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1056.

[160] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 710.

[161] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.

[162] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 710 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.

[163] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374.

[164] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.

[165] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.

[166] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 545.

[167] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.

[168] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 545 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.

[169] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249.

[170] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.

[171] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.

[172] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195.

[173] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1376.

[174] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1376.

[175] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311.

[176] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.

[177] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.

[178] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365.

[179] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.

[180] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.

[181] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527.

[182] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1071.

[183] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1071.

[184] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490.

[185] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1182.

[186] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1182.

[187] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 267:1341, 705:1056, 710:1259, 374:1111, 545:978, 249:1095, 195:1376, 311:1014, 365:1052, 527:1071 and 490:1182.

[188] In some embodiments, either or both of the primers of a primer pair composition contain at least one modified nucleobase such as 5-propynyluracil or 5-propynylcytosine for example.

[189] In some embodiments, either or both of the primers of the primer pair comprises at least one universal nucleobase such as inosine for example.

[190] In some embodiments, either or both of the primers of the primer pair comprises at least one non-templated T residue on the 5'-end.

[191] In some embodiments, either or both of the primers of the primer pair comprises at least one non-template tag.

[192] In some embodiments, either or both of the primers of the primer pair comprises at least one molecular mass modifying tag.

[193] In some embodiments, the present invention provides primers and compositions comprising pairs of primers, and kits containing the same, and methods for use in identification of bacteria. The primers are designed to produce amplification products of DNA encoding genes that have conserved and variable regions across different subgroups and genotypes of bacteria.

[194] Some embodiments are kits that contain one or more of the primer pair compositions. In some embodiments, each member of the one or more primer pairs of the kit is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from any of the primer pairs listed in Table 2.

[195] Some embodiments of the kits contain at least one calibration polynucleotide for use in quantitiation of bacteria in a given sample, and also for use as a positive control for amplification.

[196] Some embodiments of the kits contain at least one anion exchange functional group linked to a magnetic bead.

[197] In some embodiments, the present invention also provides methods for identification of bacteria. Nucleic acid from the bacterium is amplified using the primers described above to obtain an amplification product. The molecular mass of the amplification product is measured. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition is compared with a plurality of molecular masses or base compositions of known analogous bacterial identifying amplicons, wherein a match between the molecular mass or base composition and a member of the plurality of molecular masses or base compositions identifies the bacterium. In some embodiments, the molecular mass is measured by mass spectrometry in a modality such as electrospray ionization (ESI) time of flight (TOF) mass spectrometry or ESI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, for example. Other mass spectrometry techniques can also be used to measure the molecular mass of bacterial bioagent identifying amplicons.

[198] In some embodiments, the present invention is also directed to a method for determining the presence or absence of a bacterium in a sample. Nucleic acid from the sample is amplified using the composition described above to obtain an amplification product. The molecular mass of the amplification product is determined. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition of the amplification product is compared with the known molecular masses or base compositions of one or more known analogous bacterial bioagent identifying amplicons, wherein a match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of one or more known bacterial bioagent identifying amplicons indicates the presence of the bacterium in the sample. In some embodiments, the molecular mass is measured by mass spectrometry.

[199] In some embodiments, the present invention also provides methods for determination of the quantity of an unknown bacterium in a sample. The sample is contacted with the composition described above and a known quantity of a calibration polynucleotide comprising a calibration sequence. Nucleic acid from the unknown bacterium in the sample is concurrently amplified with the composition described above and nucleic acid from the calibration polynucleotide in the sample is concurrently amplified with the composition described above to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon. The molecular masses and abundances for the bacterial bioagent identifying amplicon and the calibration amplicon are determined. The bacterial bioagent identifying amplicon is distinguished from the calibration amplicon based on molecular mass and comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of bacterium in the sample. In some embodiments, the base composition of the bacterial bioagent identifying amplicon is determined.

[200] In some embodiments, the present invention provides methods for detecting or quantifying bacteria by combining a nucleic acid amplification process with a mass determination process. In some embodiments, such methods identify or otherwise analyze the bacterium by comparing mass information from an amplification product with a calibration or control product. Such methods can be carried out in a highly multiplexed and/or parallel manner allowing for the analysis of as many as 300 samples per 24 hours on a single mass measurement platform. The accuracy of the mass determination methods in some embodiments of the present invention permits allows for the ability to discriminate between different bacteria such as, for example, various genotypes and drug resistant strains of *Staphylococcus aureus*.

BRIEF DESCRIPTION OF THE DRAWINGS

[201] The foregoing summary of the invention, as well as the following detailed description of the invention, is better understood when read in conjunction with the accompanying drawings which are included by way of example and not by way of limitation.

[202] **Figure 1:** process diagram illustrating a representative primer pair selection process.

[203] **Figure 2:** process diagram illustrating an embodiment of the calibration method.

[204] **Figure 3:** common pathogenic bacteria and primer pair coverage. The primer pair number in the upper right hand corner of each polygon indicates that the primer pair can produce a bioagent identifying amplicon for all species within that polygon.

[205] **Figure 4**: a representative 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples (labeled NHRC samples) closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[206] **Figure 5**: a representative mass spectrum of amplification products indicating the presence of bioagent identifying amplicons of *Streptococcus pyogenes*, *Neisseria* meningitidis, and *Haemophilus influenzae* obtained from amplification of nucleic acid from a clinical sample with primer pair number 349 which targets 23S rRNA. Experimentally determined molecular masses and base compositions for the sense strand of each amplification product are shown.

[207] **Figure 6**: a representative mass spectrum of amplification products representing a bioagent identifying amplicon of *Streptococcus pyogenes*, and a calibration amplicon obtained from amplification of nucleic acid from a clinical sample with primer pair number 356 which targets rplB. The experimentally determined molecular mass and base composition for the sense strand of the *Streptococcus pyogenes* amplification product is shown.

[208] **Figure 7**: a representative mass spectrum of an amplified nucleic acid mixture which contained the Ames strain of *Bacillus anthracis*, a known quantity of combination calibration polynucleotide (SEQ ID NO: 1464), and primer pair number 350 which targets the capC gene on

the virulence plasmid pX02 of *Bacillus anthracis*. Calibration amplicons produced in the amplification reaction are visible in the mass spectrum as indicated and abundance data (peak height) are used to calculate the quantity of the Ames strain of *Bacillus anthracis*.

DEFINITIONS

[209] As used herein, the term "abundance" refers to an amount. The amount may be described in terms of concentration which are common in molecular biology such as "copy number," "pfu or plate-forming unit" which are well known to those with ordinary skill. Concentration may be relative to a known standard or may be absolute.

[210] As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" also comprises "sample template."

[211] As used herein the term "amplification" refers to a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (i.e., replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (i.e., synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out. Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Qß replicase, MDV-1 RNA is the specific template for the replicase (D.L. Kacian et al., Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin et al., Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (D.Y. Wu and R. B. Wallace, Genomics 4:560 [1989]). Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), PCR Technology, Stockton Press [1989]).

[212] As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification, excluding primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

[213] As used herein, the term “analogous” when used in context of comparison of bioagent identifying amplicons indicates that the bioagent identifying amplicons being compared are produced with the same pair of primers. For example, bioagent identifying amplicon “A” and bioagent identifying amplicon “B”, produced with the same pair of primers are analogous with respect to each other. Bioagent identifying amplicon “C”, produced with a different pair of primers is not analogous to either bioagent identifying amplicon “A” or bioagent identifying amplicon “B”.

[214] As used herein, the term “anion exchange functional group” refers to a positively charged functional group capable of binding an anion through an electrostatic interaction. The most well known anion exchange functional groups are the amines, including primary, secondary, tertiary and quaternary amines.

[215] The term "bacteria" or “bacterium” refers to any member of the groups of eubacteria and archaebacteria.

[216] As used herein, a “base composition” is the exact number of each nucleobase (for example, A, T, C and G) in a segment of nucleic acid. For example, amplification of nucleic acid of *Staphylococcus aureus* strain carrying the lukS-PV gene with primer pair number 2095 (SEQ ID NOs: 456:1261) produces an amplification product 117 nucleobases in length from nucleic acid of the lukS-PV gene that has a base composition of A35 G17 C19 T46 (by convention - with reference to the sense strand of the amplification product). Because the molecular masses of each of the four natural nucleotides and chemical modifications thereof are known (if applicable), a measured molecular mass can be deconvoluted to a list of possible base compositions. Identification of a base composition of a sense strand which is complementary to the corresponding antisense strand in terms of base composition provides a confirmation of the true base composition of an unknown amplification product. For example, the base composition of the antisense strand of the 139 nucleobase amplification product described above is A46 G19 C17 T35.

[217] As used herein, a “base composition probability cloud” is a representation of the diversity in base composition resulting from a variation in sequence that occurs among different isolates of a given

species. The "base composition probability cloud" represents the base composition constraints for each species and is typically visualized using a pseudo four-dimensional plot.

[218] In the context of this invention, a "bioagent" is any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus. Examples of bioagents include, but are not limited, to cells, (including but not limited to human clinical samples, bacterial cells and other pathogens), viruses, fungi, protists, parasites, and pathogenicity markers (including but not limited to: pathogenicity islands, antibiotic resistance genes, virulence factors, toxin genes and other bioregulating compounds). Samples may be alive or dead or in a vegetative state (for example, vegetative bacteria or spores) and may be encapsulated or bioengineered. In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[219] As used herein, a "bioagent division" is defined as group of bioagents above the species level and includes but is not limited to, orders, families, classes, clades, genera or other such groupings of bioagents above the species level.

[220] As used herein, the term "bioagent identifying amplicon" refers to a polynucleotide that is amplified from a bioagent in an amplification reaction and which 1) provides sufficient variability to distinguish among bioagents from whose nucleic acid the bioagent identifying amplicon is produced and 2) whose molecular mass is amenable to a rapid and convenient molecular mass determination modality such as mass spectrometry, for example.

[221] As used herein, the term "biological product" refers to any product originating from an organism. Biological products are often products of processes of biotechnology. Examples of biological products include, but are not limited to: cultured cell lines, cellular components, antibodies, proteins and other cell-derived biomolecules, growth media, growth harvest fluids, natural products and bio-pharmaceutical products.

[222] The terms "biowarfare agent" and "bioweapon" are synonymous and refer to a bacterium, virus, fungus or protozoan that could be deployed as a weapon to cause bodily harm to individuals. Military or terrorist groups may be implicated in deployment of biowarfare agents.

[223] In context of this invention, the term "broad range survey primer pair" refers to a primer pair designed to produce bioagent identifying amplicons across different broad groupings of bioagents. For example, the ribosomal RNA-targeted primer pairs are broad range survey primer pairs which have the capability of producing bacterial bioagent identifying amplicons for essentially all known bacteria. With

respect to broad range primer pairs employed for identification of bacteria, a broad range survey primer pair for bacteria such as 16S rRNA primer pair number 346 (SEQ ID NOs: 202:1110) for example, will produce an bacterial bioagent identifying amplicon for essentially all known bacteria.

[224] The term "calibration amplicon" refers to a nucleic acid segment representing an amplification product obtained by amplification of a calibration sequence with a pair of primers designed to produce a bioagent identifying amplicon.

[225] The term "calibration sequence" refers to a polynucleotide sequence to which a given pair of primers hybridizes for the purpose of producing an internal (i.e: included in the reaction) calibration standard amplification product for use in determining the quantity of a bioagent in a sample. The calibration sequence may be expressly added to an amplification reaction, or may already be present in the sample prior to analysis.

[226] The term "clade primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for species belonging to a clade group. A clade primer pair may also be considered as a "speciating" primer pair which is useful for distinguishing among closely related species.

[227] The term "codon" refers to a set of three adjoined nucleotides (triplet) that codes for an amino acid or a termination signal.

[228] In context of this invention, the term "codon base composition analysis," refers to determination of the base composition of an individual codon by obtaining a bioagent identifying amplicon that includes the codon. The bioagent identifying amplicon will at least include regions of the target nucleic acid sequence to which the primers hybridize for generation of the bioagent identifying amplicon as well as the codon being analyzed, located between the two primer hybridization regions.

[229] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) related by the base-pairing rules. For example, for the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids. Either term may also be used in reference to individual nucleotides, especially within the

context of polynucleotides. For example, a particular nucleotide within an oligonucleotide may be noted for its complementarity, or lack thereof, to a nucleotide within another nucleic acid strand, in contrast or comparison to the complementarity between the rest of the oligonucleotide and the nucleic acid strand.

[230] The term "complement of a nucleic acid sequence" as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other, is in "antiparallel association." Certain bases not commonly found in natural nucleic acids may be included in the nucleic acids of the present invention and include, for example, inosine and 7-deazaguanine. Complementarity need not be perfect; stable duplexes may contain mismatched base pairs or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. Where a first oligonucleotide is complementary to a region of a target nucleic acid and a second oligonucleotide has complementary to the same region (or a portion of this region) a "region of overlap" exists along the target nucleic acid. The degree of overlap will vary depending upon the extent of the complementarity.

[231] In context of this invention, the term "division-wide primer pair" refers to a primer pair designed to produce bioagent identifying amplicons within sections of a broader spectrum of bioagents For example, primer pair number 352 (SEQ ID NOs: 687:1411), a division-wide primer pair, is designed to produce bacterial bioagent identifying amplicons for members of the Bacillus group of bacteria which comprises, for example, members of the genera *Streptococci, Enterococci,* and *Staphylococci*. Other division-wide primer pairs may be used to produce bacterial bioagent identifying amplicons for other groups of bacterial bioagents.

[232] As used herein, the term "concurrently amplifying" used with respect to more than one amplification reaction refers to the act of simultaneously amplifying more than one nucleic acid in a single reaction mixture.

[233] As used herein, the term "drill-down primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for identification of sub-species characteristics or confirmation of a species assignment. For example, primer pair number 2146 (SEQ ID NOs: 437:1137), a drill-down *Staphylococcus aureus* genotyping primer pair, is designed to produce *Staphylococcus aureus* genotyping amplicons. Other drill-down primer pairs may be used to produce bioagent identifying amplicons for *Staphylococcus aureus* and other bacterial species.

[234] The term "duplex" refers to the state of nucleic acids in which the base portions of the nucleotides on one strand are bound through hydrogen bonding the their complementary bases arrayed on a second strand. The condition of being in a duplex form reflects on the state of the bases of a nucleic acid. By virtue of base pairing, the strands of nucleic acid also generally assume the tertiary structure of a double helix, having a major and a minor groove. The assumption of the helical form is implicit in the act of becoming duplexed.

[235] As used herein, the term “etiology” refers to the causes or origins, of diseases or abnormal physiological conditions.

[236] The term "gene" refers to a DNA sequence that comprises control and coding sequences necessary for the production of an RNA having a non-coding function (e.g., a ribosomal or transfer RNA), a polypeptide or a precursor. The RNA or polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or function is retained.

[237] The terms "homology," "homologous" and “sequence identity” refer to a degree of identity. There may be partial homology or complete homology. A partially homologous sequence is one that is less than 100% identical to another sequence. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is otherwise identical to another 20 nucleobase primer but having two non-identical residues has 18 of 20 identical residues (18/20 = 0.9 or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of a primer 20 nucleobases in length would have 15/20 = 0.75 or 75% sequence identity with the 20 nucleobase primer. In context of the present invention, sequence identity is meant to be properly determined when the query sequence and the subject sequence are both described and aligned in the 5’ to 3’ direction. Sequence alignment algorithms such as BLAST, will return results in two different alignment orientations. In the Plus/Plus orientation, both the query sequence and the subject sequence are aligned in the 5’ to 3’ direction. On the other hand, in the Plus/Minus orientation, the query sequence is in the 5’ to 3’ direction while the subject sequence is in the 3’ to 5’ direction. It should be understood that with respect to the primers of the present invention, sequence identity is properly determined when the alignment is designated as Plus/Plus. Sequence identity may also encompass alternate or modified nucleobases that perform in a functionally similar manner to the regular nucleobases adenine, thymine, guanine and cytosine with respect to hybridization and primer extension in amplification reactions. In a non-limiting example, if the 5-propynyl pyrimidines propyne C and/or propyne T replace one or more C or T residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. In another non-limiting example, Inosine (I) may be used as a replacement for G or T and effectively

hybridize to C, A or U (uracil). Thus, if inosine replaces one or more C, A or U residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. Other such modified or universal bases may exist which would perform in a functionally similar manner for hybridization and amplification reactions and will be understood to fall within this definition of sequence identity.

[238] As used herein, "housekeeping gene" refers to a gene encoding a protein or RNA involved in basic functions required for survival and reproduction of a bioagent. Housekeeping genes include, but are not limited to genes encoding RNA or proteins involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, secretion and the like.

[239] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, and the T_m of the formed hybrid. "Hybridization" methods involve the annealing of one nucleic acid to another, complementary nucleic acid, i.e., a nucleic acid having a complementary nucleotide sequence. The ability of two polymers of nucleic acid containing complementary sequences to find each other and anneal through base pairing interaction is a well-recognized phenomenon. The initial observations of the "hybridization" process by Marmur and Lane, Proc. Natl. Acad. Sci. USA 46:453 (1960) and Doty et al., Proc. Natl. Acad. Sci. USA 46:461 (1960) have been followed by the refinement of this process into an essential tool of modem biology.

[240] The term "*in silico*" refers to processes taking place via computer calculations. For example, electronic PCR (ePCR) is a process analogous to ordinary PCR except that it is carried out using nucleic acid sequences and primer pair sequences stored on a computer formatted medium.

[241] As used herein, "intelligent primers" are primers that are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and, upon amplification, yield amplification products which ideally provide enough variability to distinguish individual bioagents, and which are amenable to molecular mass analysis. By the term "highly conserved," it is meant that the sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity among all, or at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of species or strains.

[242] The “ligase chain reaction” (LCR; sometimes referred to as "Ligase Amplification Reaction" (LAR) described by Barany, Proc. Natl. Acad. Sci., 88:189 (1991); Barany, PCR Methods and Applic., 1:5 (1991); and Wu and Wallace, Genomics 4:560 (1989) has developed into a well-recognized alternative method for amplifying nucleic acids. In LCR, four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, that hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules. Importantly, in LCR, two probes are ligated together only when they base-pair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, hybridization and ligation amplify a short segment of DNA. LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes. However, because the four oligonucleotides used in this assay can pair to form two short ligatable fragments, there is the potential for the generation of target-independent background signal. The use of LCR for mutant screening is limited to the examination of specific nucleic acid positions.

[243] The term "locked nucleic acid" or "LNA" refers to a nucleic acid analogue containing one or more 2'-O, 4'-C-methylene-β-D-ribofuranosyl nucleotide monomers in an RNA mimicking sugar conformation. LNA oligonucleotides display unprecedented hybridization affinity toward complementary single-stranded RNA and complementary single- or double-stranded DNA. LNA oligonucleotides induce A-type (RNA-like) duplex conformations. The primers of the present invention may contain LNA modifications.

[244] As used herein, the term "mass-modifying tag" refers to any modification to a given nucleotide which results in an increase in mass relative to the analogous non-mass modified nucleotide. Mass-modifying tags can include heavy isotopes of one or more elements included in the nucleotide such as carbon-13 for example. Other possible modifications include addition of substituents such as iodine or bromine at the 5 position of the nucleobase for example.

[245] The term "mass spectrometry" refers to measurement of the mass of atoms or molecules. The molecules are first converted to ions, which are separated using electric or magnetic fields according to the ratio of their mass to electric charge. The measured masses are used to identity the molecules.

[246] The term "microorganism" as used herein means an organism too small to be observed with the unaided eye and includes, but is not limited to bacteria, virus, protozoans, fungi; and ciliates.

[247] The term "multi-drug resistant" or multiple-drug resistant" refers to a microorganism which is resistant to more than one of the antibiotics or antimicrobial agents used in the treatment of said microorganism.

[248] The term "multiplex PCR" refers to a PCR reaction where more than one primer set is included in the reaction pool allowing 2 or more different DNA targets to be amplified by PCR in a single reaction tube.

[249] The term "non-template tag" refers to a stretch of at least three guanine or cytosine nucleobases of a primer used to produce a bioagent identifying amplicon which are not complementary to the template. A non-template tag is incorporated into a primer for the purpose of increasing the primer-duplex stability of later cycles of amplification by incorporation of extra G-C pairs which each have one additional hydrogen bond relative to an A-T pair.

[250] The term "nucleic acid sequence" as used herein refers to the linear composition of the nucleic acid residues A, T, C or G or any modifications thereof, within an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single or double stranded, and represent the sense or antisense strand

[251] As used herein, the term “nucleobase” is synonymous with other terms in use in the art including “nucleotide,” “deoxynucleotide,” “nucleotide residue,” “deoxynucleotide residue,” “nucleotide triphosphate (NTP),” or deoxynucleotide triphosphate (dNTP).

[252] The term "nucleotide analog" as used herein refers to modified or non-naturally occurring nucleotides such as 5-propynyl pyrimidines (i.e., 5-propynyl-dTTP and 5-propynyl-dTCP), 7-deaza purines (i.e., 7-deaza-dATP and 7-deaza-dGTP). Nucleotide analogs include base analogs and comprise modified forms of deoxyribonucleotides as well as ribonucleotides.

[253] The term "oligonucleotide" as used herein is defined as a molecule comprising two or more deoxyribonucleotides or ribonucleotides, preferably at least 5 nucleotides, more preferably at least about 13 to 35 nucleotides. The exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, PCR, or a combination thereof. Because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage, an end of an oligonucleotide is referred to as the "5’-end" if its 5' phosphate is not

linked to the 3' oxygen of a mononucleotide pentose ring and as the "3'-end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. A first region along a nucleic acid strand is said to be upstream of another region if the 3' end of the first region is before the 5' end of the second region when moving along a strand of nucleic acid in a 5' to 3' direction. All oligonucleotide primers disclosed herein are understood to be presented in the 5' to 3' direction when reading left to right. When two different, non-overlapping oligonucleotides anneal to different regions of the same linear complementary nucleic acid sequence, and the 3' end of one oligonucleotide points towards the 5' end of the other, the former may be called the "upstream" oligonucleotide and the latter the "downstream" oligonucleotide. Similarly, when two overlapping oligonucleotides are hybridized to the same linear complementary nucleic acid sequence, with the first oligonucleotide positioned such that its 5' end is upstream of the 5' end of the second oligonucleotide, and the 3' end of the first oligonucleotide is upstream of the 3' end of the second oligonucleotide, the first oligonucleotide may be called the "upstream" oligonucleotide and the second oligonucleotide may be called the "downstream" oligonucleotide.

[254] In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[255] As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

[256] The term "peptide nucleic acid" ("PNA") as used herein refers to a molecule comprising bases or base analogs such as would be found in natural nucleic acid, but attached to a peptide backbone rather than the sugar-phosphate backbone typical of nucleic acids. The attachment of the bases to the peptide is such as to allow the bases to base pair with complementary bases of nucleic acid in a manner similar to that of an oligonucleotide. These small molecules, also designated anti gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, et al. Anticancer Drug Des. 8:53 63). The primers of the present invention may comprise PNAs.

[257] The term "polymerase" refers to an enzyme having the ability to synthesize a complementary strand of nucleic acid from a starting template nucleic acid strand and free dNTPs.

[258] As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that

describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified." With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (e.g., hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of 32P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

[259] The term "polymerization means" or "polymerization agent" refers to any agent capable of facilitating the addition of nucleoside triphosphates to an oligonucleotide. Preferred polymerization means comprise DNA and RNA polymerases.

[260] As used herein, the terms "pair of primers," or "primer pair" are synonymous. A primer pair is used for amplification of a nucleic acid sequence. A pair of primers comprises a forward primer and a reverse primer. The forward primer hybridizes to a sense strand of a target gene sequence to be amplified and primes synthesis of an antisense strand (complementary to the sense strand) using the target sequence as a template. A reverse primer hybridizes to the antisense strand of a target gene sequence to be amplified and primes synthesis of a sense strand (complementary to the antisense strand) using the target sequence as a template.

[261] The primers are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which ideally provide enough variability to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus design of the primers requires selection of a variable region with appropriate variability to resolve the identity of a given bioagent. Bioagent identifying amplicons are ideally specific to the identity of the bioagent.

[262] Properties of the primers may include any number of properties related to structure including, but not limited to: nucleobase length which may be contiguous (linked together) or non-contiguous (for example, two or more contiguous segments which are joined by a linker or loop moiety), modified or universal nucleobases (used for specific purposes such as for example, increasing hybridization affinity, preventing non-templated adenylation and modifying molecular mass) percent complementarity to a given target sequences.

[263] Properties of the primers also include functional features including, but not limited to, orientation of hybridization (forward or reverse) relative to a nucleic acid template. The coding or sense strand is the strand to which the forward priming primer hybridizes (forward priming orientation) while the reverse priming primer hybridizes to the non-coding or antisense strand (reverse priming orientation). The functional properties of a given primer pair also include the generic template nucleic acid to which the primer pair hybridizes. For example, identification of bioagents can be accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Other primers may have the functionality of producing bioagent identifying amplicons for members of a given taxonomic genus, clade, species, sub-species or genotype (including genetic variants which may include presence of virulence genes or antibiotic resistance genes or mutations). Additional functional properties of primer pairs include the functionality of performing amplification either singly (single primer pair per amplification reaction vessel) or in a multiplex fashion (multiple primer pairs and multiple amplification reactions within a single reaction vessel).

[264] As used herein, the terms "purified" or "substantially purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated,

and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. An "isolated polynucleotide" or "isolated oligonucleotide" is therefore a substantially purified polynucleotide.

[265] The term "reverse transcriptase" refers to an enzyme having the ability to transcribe DNA from an RNA template. This enzymatic activity is known as reverse transcriptase activity. Reverse transcriptase activity is desirable in order to obtain DNA from RNA viruses which can then be amplified and analyzed by the methods of the present invention.

[266] The term "ribosomal RNA" or "rRNA" refers to the primary ribonucleic acid constituent of ribosomes. Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm. Ribosomal RNAs are transcribed from the DNA genes encoding them.

[267] The term "sample" in the present specification and claims is used in its broadest sense. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures). On the other hand, it is meant to include both biological and environmental samples. A sample may include a specimen of synthetic origin. Biological samples may be animal, including human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may be obtained from all of the various families of domestic animals, as well as feral or wild animals, including, but not limited to, such animals as ungulates, bear, fish, lagamorphs, rodents, etc. Environmental samples include environmental material such as surface matter, soil, water, air and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention. The term "source of target nucleic acid" refers to any sample that contains nucleic acids (RNA or DNA). Particularly preferred sources of target nucleic acids are biological samples including, but not limited to blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen.

[268] As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is often a contaminant. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

[269] A "segment" is defined herein as a region of nucleic acid within a target sequence.

[270] The “self-sustained sequence replication reaction” (3SR) (Guatelli et al., Proc. Natl. Acad. Sci., 87:1874-1878 [1990], with an erratum at Proc. Natl. Acad. Sci., 87:7797 [1990]) is a transcription-based *in vitro* amplification system (Kwok et al., Proc. Natl. Acad. Sci., 86:1173-1177 [1989]) that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection (Fahy et al., PCR Meth. Appl., 1:25-33 [1991]). In this method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5′ end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest. The use of 3SR to detect mutations is kinetically limited to screening small segments of DNA (e.g., 200-300 base pairs).

[271] As used herein, the term ""sequence alignment"" refers to a listing of multiple DNA or amino acid sequences and aligns them to highlight their similarities. The listings can be made using bioinformatics computer programs.

[272] In context of this invention, the term "speciating primer pair" refers to a primer pair designed to produce a bioagent identifying amplicon with the diagnostic capability of identifying species members of a group of genera or a particular genus of bioagents. Primer pair number 2249 (SEQ ID NOs: 430:1321), for example, is a speciating primer pair used to distinguish *Staphylococcus aureus* from other species of the genus *Staphylococcus*.

[273] As used herein, a “sub-species characteristic” is a genetic characteristic that provides the means to distinguish two members of the same bioagent species. For example, one viral strain could be distinguished from another viral strain of the same species by possessing a genetic change (e.g., for example, a nucleotide deletion, addition or substitution) in one of the viral genes, such as the RNA-dependent RNA polymerase. Sub-species characteristics such as virulence genes and drug-are responsible for the phenotypic differences among the different strains of bacteria.

[274] As used herein, the term "target" is used in a broad sense to indicate the gene or genomic region being amplified by the primers. Because the present invention provides a plurality of amplification products from any given primer pair (depending on the bioagent being analyzed), multiple amplification products from different specific nucleic acid sequences may be obtained. Thus, the term "target" is not used to refer to a single specific nucleic acid sequence. The "target" is sought to be sorted out from other nucleic acid sequences and contains a sequence that has at least partial complementarity with an

oligonucleotide primer. The target nucleic acid may comprise single- or double-stranded DNA or RNA. A "segment" is defined as a region of nucleic acid within the target sequence.

[275] The term "template" refers to a strand of nucleic acid on which a complementary copy is built from nucleoside triphosphates through the activity of a template-dependent nucleic acid polymerase. Within a duplex the template strand is, by convention, depicted and described as the "bottom" strand. Similarly, the non-template strand is often depicted and described as the "top" strand.

[276] As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: T_m=81.5+0.41(% G+C), when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985). Other references (e.g., Allawi, H. T. & SantaLucia, J., Jr. Thermodynamics and NMR of internal G.T mismatches in DNA. Biochemistry 36, 10581-94 (1997) include more sophisticated computations which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.

[277] The term “triangulation genotyping analysis” refers to a method of genotyping a bioagent by measurement of molecular masses or base compositions of amplification products, corresponding to bioagent identifying amplicons, obtained by amplification of regions of more than one gene. In this sense, the term “triangulation” refers to a method of establishing the accuracy of information by comparing three or more types of independent points of view bearing on the same findings. Triangulation genotyping analysis carried out with a plurality of triangulation genotyping analysis primers yields a plurality of base compositions that then provide a pattern or "barcode" from which a species type can be assigned. The species type may represent a previously known sub-species or strain, or may be a previously unknown strain having a specific and previously unobserved base composition barcode indicating the existence of a previously unknown genotype.

[278] As used herein, the term "triangulation genotyping analysis primer pair" is a primer pair designed to produce bioagent identifying amplicons for determining species types in a triangulation genotyping analysis.

[279] The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as “triangulation identification.” Triangulation identification is pursued by

analyzing a plurality of bioagent identifying amplicons produced with different primer pairs. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[280] In the context of this invention, the term "unknown bioagent" may mean either: (i) a bioagent whose existence is known (such as the well known bacterial species *Staphylococcus aureus* for example) but which is not known to be in a sample to be analyzed, or (ii) a bioagent whose existence is not known (for example, the SARS coronavirus was unknown prior to April 2003). For example, if the method for identification of coronaviruses disclosed in commonly owned U.S. Patent Serial No. 10/829,826 (incorporated herein by reference in its entirety) was to be employed prior to April 2003 to identify the SARS coronavirus in a clinical sample, both meanings of "unknown" bioagent are applicable since the SARS coronavirus was unknown to science prior to April, 2003 and since it was not known what bioagent (in this case a coronavirus) was present in the sample. On the other hand, if the method of U.S. Patent Serial No. 10/829,826 was to be employed subsequent to April 2003 to identify the SARS coronavirus in a clinical sample, only the first meaning (i) of "unknown" bioagent would apply since the SARS coronavirus became known to science subsequent to April 2003 and since it was not known what bioagent was present in the sample.

[281] The term "variable sequence" as used herein refers to differences in nucleic acid sequence between two nucleic acids. For example, the genes of two different bacterial species may vary in sequence by the presence of single base substitutions and/or deletions or insertions of one or more nucleotides. These two forms of the structural gene are said to vary in sequence from one another. In the context of the present invention, "viral nucleic acid" includes, but is not limited to, DNA, RNA, or DNA that has been obtained from viral RNA, such as, for example, by performing a reverse transcription reaction. Viral RNA can either be single-stranded (of positive or negative polarity) or double-stranded.

[282] The term "virus" refers to obligate, ultramicroscopic, parasites that are incapable of autonomous replication (i.e., replication requires the use of the host cell's machinery). Viruses can survive outside of a host cell but cannot replicate.

[283] The term "wild-type" refers to a gene or a gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified", "mutant" or "polymorphic" refers to a gene or gene product

that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[284] As used herein, a "wobble base" is a variation in a codon found at the third nucleotide position of a DNA triplet. Variations in conserved regions of sequence are often found at the third nucleotide position due to redundancy in the amino acid code.

DETAILED DESCRIPTION OF EMBODIMENTS

A. Bioagent Identifying Amplicons

[285] The present invention provides methods for detection and identification of unknown bioagents using bioagent identifying amplicons. Primers are selected to hybridize to conserved sequence regions of nucleic acids derived from a bioagent, and which bracket variable sequence regions to yield a bioagent identifying amplicon, which can be amplified and which is amenable to molecular mass determination. The molecular mass then provides a means to uniquely identify the bioagent without a requirement for prior knowledge of the possible identity of the bioagent. The molecular mass or corresponding base composition signature of the amplification product is then matched against a database of molecular masses or base composition signatures. A match is obtained when an experimentally-determined molecular mass or base composition of an analyzed amplification product is compared with known molecular masses or base compositions of known bioagent identifying amplicons and the experimentally determined molecular mass or base composition is the same as the molecular mass or base composition of one of the known bioagent identifying amplicons. Alternatively, the experimentally-determined molecular mass or base composition may be within experimental error of the molecular mass or base composition of a known bioagent identifying amplicon and still be classified as a match. In some cases, the match may also be classified using a probability of match model such as the models described in U.S. Serial No. 11/073,362, which is commonly owned and incorporated herein by reference in entirety. Furthermore, the method can be applied to rapid parallel multiplex analyses, the results of which can be employed in a triangulation identification strategy. The present method provides rapid throughput and does not require nucleic acid sequencing of the amplified target sequence for bioagent detection and identification.

[286] Despite enormous biological diversity, all forms of life on earth share sets of essential, common features in their genomes. Since genetic data provide the underlying basis for identification of bioagents by the methods of the present invention, it is necessary to select segments of nucleic acids which ideally provide enough variability to distinguish each individual bioagent and whose molecular mass is amenable to molecular mass determination.

[287] Unlike bacterial genomes, which exhibit conservation of numerous genes (i.e. housekeeping genes) across all organisms, viruses do not share a gene that is essential and conserved among all virus families. Therefore, viral identification is achieved within smaller groups of related viruses, such as members of a particular virus family or genus. For example, RNA-dependent RNA polymerase is present in all single-stranded RNA viruses and can be used for broad priming as well as resolution within the virus family.

[288] In some embodiments of the present invention, at least one bacterial nucleic acid segment is amplified in the process of identifying the bacterial bioagent. Thus, the nucleic acid segments that can be amplified by the primers disclosed herein and that provide enough variability to distinguish each individual bioagent and whose molecular masses are amenable to molecular mass determination are herein described as bioagent identifying amplicons.

[289] In some embodiments of the present invention, bioagent identifying amplicons comprise from about 45 to about 150 nucleobases (i.e. from about 45 to about 200 linked nucleosides), although both longer and short regions may be used. One of ordinary skill in the art will appreciate that the invention embodies compounds of 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleobases in length, or any range therewithin.

[290] It is the combination of the portions of the bioagent nucleic acid segment to which the primers hybridize (hybridization sites) and the variable region between the primer hybridization sites that comprises the bioagent identifying amplicon. Thus, it can be said that a given bioagent identifying amplicon is "defined by" a given pair of primers.

[291] In some embodiments, bioagent identifying amplicons amenable to molecular mass determination which are produced by the primers described herein are either of a length, size or mass compatible with the particular mode of molecular mass determination or compatible with a means of providing a predictable fragmentation pattern in order to obtain predictable fragments of a length compatible with the particular mode of molecular mass determination. Such means of providing a predictable fragmentation pattern of an amplification product include, but are not limited to, cleavage with chemical reagents, restriction enzymes or cleavage primers, for example. Thus, in some

embodiments, bioagent identifying amplicons are larger than 150 nucleobases and are amenable to molecular mass determination following restriction digestion. Methods of using restriction enzymes and cleavage primers are well known to those with ordinary skill in the art.

[292] In some embodiments, amplification products corresponding to bioagent identifying amplicons are obtained using the polymerase chain reaction (PCR) that is a routine method to those with ordinary skill in the molecular biology arts. Other amplification methods may be used such as ligase chain reaction (LCR), low-stringency single primer PCR, and multiple strand displacement amplification (MDA). These methods are also known to those with ordinary skill.

B. Primers and Primer Pairs

[293] In some embodiments, the primers are designed to bind to conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which provide variability sufficient to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus, design of the primers involves selection of a variable region with sufficient variability to resolve the identity of a given bioagent. In some embodiments, bioagent identifying amplicons are specific to the identity of the bioagent.

[294] In some embodiments, identification of bioagents is accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Examples of broad range survey primers include, but are not limited to: primer pair numbers: 346 (SEQ ID NOs: 202:1110), 347 (SEQ ID NOs: 560:1278), 348 SEQ ID NOs: 706:895), and 361 (SEQ ID NOs: 697:1398) which target DNA encoding 16S rRNA, and primer pair numbers 349 (SEQ ID NOs: 401:1156) and 360 (SEQ ID NOs: 409:1434) which target DNA encoding 23S rRNA.

[295] In some embodiments, drill-down primers are designed with the objective of identifying a bioagent at the sub-species level (including strains, subtypes, variants and isolates) based on sub-species characteristics which may, for example, include single nucleotide polymorphisms (SNPs), variable

number tandem repeats (VNTRs), deletions, drug resistance mutations or any other modification of a nucleic acid sequence of a bioagent relative to other members of a species having different sub-species characteristics. Drill-down intelligent primers are not always required for identification at the sub-species level because broad range survey intelligent primers may, in some cases provide sufficient identification resolution to accomplishing this identification objective. Examples of drill-down primers include, but are not limited to: confirmation primer pairs such as primer pair numbers 351 (SEQ ID NOs: 355:1423) and 353 (SEQ ID NOs: 220:1394), which target the pX01 virulence plasmid of *Bacillus anthracis*. Other examples of drill-down primer pairs are found in sets of triangulation genotyping primer pairs such as, for example, the primer pair number 2146 (SEQ ID NOs: 437:1137) which targets the arcC gene (encoding carmabate kinase) and is included in an 8 primer pair panel or kit for use in genotyping *Staphylococcus aureus*, or in other panels or kits of primer pairs used for determining drug-resistant bacterial strains, such as, for example, primer pair number 2095 (SEQ ID NOs: 456:1261) which targets the pv-luk gene (encoding Panton-Valentine leukocidin) and is included in an 8 primer pair panel or kit for use in identification of drug resistant strains of *Staphylococcus aureus*.

[296] A representative process flow diagram used for primer selection and validation process is outlined in Figure 1. For each group of organisms, candidate target sequences are identified **(200)** from which nucleotide alignments are created **(210)** and analyzed **(220)**. Primers are then designed by selecting appropriate priming regions **(230)** to facilitate the selection of candidate primer pairs **(240)**. The primer pairs are then subjected to *in silico* analysis by electronic PCR (ePCR) **(300)** wherein bioagent identifying amplicons are obtained from sequence databases such as GenBank or other sequence collections **(310)** and checked for specificity *in silico* **(320)**. Bioagent identifying amplicons obtained from GenBank sequences **(310)** can also be analyzed by a probability model which predicts the capability of a given amplicon to identify unknown bioagents such that the base compositions of amplicons with favorable probability scores are then stored in a base composition database **(325)**. Alternatively, base compositions of the bioagent identifying amplicons obtained from the primers and GenBank sequences can be directly entered into the base composition database **(330)**. Candidate primer pairs **(240)** are validated by testing their ability to hybridize to target nucleic acid by an *in* vitro amplification by a method such as PCR analysis **(400)** of nucleic acid from a collection of organisms **(410)**. Amplification products thus obtained are analyzed by gel electrophoresis or by mass spectrometry to confirm the sensitivity, specificity and reproducibility of the primers used to obtain the amplification products **(420)**.

[297] Many of the important pathogens, including the organisms of greatest concern as biowarfare agents, have been completely sequenced. This effort has greatly facilitated the design of primers for the detection of unknown bioagents. The combination of broad-range priming with division-wide and drill-down priming has been used very successfully in several applications of the technology, including

environmental surveillance for biowarfare threat agents and clinical sample analysis for medically important pathogens.

[298] Synthesis of primers is well known and routine in the art. The primers may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

[299] In some embodiments primers are employed as compositions for use in methods for identification of bacterial bioagents as follows: a primer pair composition is contacted with nucleic acid (such as, for example, bacterial DNA or DNA reverse transcribed from the rRNA) of an unknown bacterial bioagent. The nucleic acid is then amplified by a nucleic acid amplification technique, such as PCR for example, to obtain an amplification product that represents a bioagent identifying amplicon. The molecular mass of each strand of the double-stranded amplification product is determined by a molecular mass measurement technique such as mass spectrometry for example, wherein the two strands of the double-stranded amplification product are separated during the ionization process. In some embodiments, the mass spectrometry is electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) or electrospray time of flight mass spectrometry (ESI-TOF-MS). A list of possible base compositions can be generated for the molecular mass value obtained for each strand and the choice of the correct base composition from the list is facilitated by matching the base composition of one strand with a complementary base composition of the other strand. The molecular mass or base composition thus determined is then compared with a database of molecular masses or base compositions of analogous bioagent identifying amplicons for known viral bioagents. A match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of an analogous bioagent identifying amplicon for a known viral bioagent indicates the identity of the unknown bioagent. In some embodiments, the primer pair used is one of the primer pairs of Table 2. In some embodiments, the method is repeated using one or more different primer pairs to resolve possible ambiguities in the identification process or to improve the confidence level for the identification assignment.

[300] In some embodiments, a bioagent identifying amplicon may be produced using only a single primer (either the forward or reverse primer of any given primer pair), provided an appropriate amplification method is chosen, such as, for example, low stringency single primer PCR (LSSP-PCR). Adaptation of this amplification method in order to produce bioagent identifying amplicons can be accomplished by one with ordinary skill in the art without undue experimentation.

[301] In some embodiments, the oligonucleotide primers are broad range survey primers which hybridize to conserved regions of nucleic acid encoding the hexon gene of all (or between 80% and 100%, between 85% and 100%, between 90% and 100% or between 95% and 100%) known bacteria and produce bacterial bioagent identifying amplicons.

[302] In some cases, the molecular mass or base composition of a bacterial bioagent identifying amplicon defined by a broad range survey primer pair does not provide enough resolution to unambiguously identify a bacterial bioagent at or below the species level. These cases benefit from further analysis of one or more bacterial bioagent identifying amplicons generated from at least one additional broad range survey primer pair or from at least one additional division-wide primer pair. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as triangulation identification.

[303] In other embodiments, the oligonucleotide primers are division-wide primers which hybridize to nucleic acid encoding genes of species within a genus of bacteria. In other embodiments, the oligonucleotide primers are drill-down primers which enable the identification of sub-species characteristics. Drill down primers provide the functionality of producing bioagent identifying amplicons for drill-down analyses such as strain typing when contacted with nucleic acid under amplification conditions. Identification of such sub-species characteristics is often critical for determining proper clinical treatment of viral infections. In some embodiments, sub-species characteristics are identified using only broad range survey primers and division-wide and drill-down primers are not used.

[304] In some embodiments, the primers used for amplification hybridize to and amplify genomic DNA, and DNA of bacterial plasmids.

[305] In some embodiments, various computer software programs may be used to aid in design of primers for amplification reactions such as *Primer Premier 5* (Premier Biosoft, Palo Alto, CA) or *OLIGO* Primer Analysis Software (Molecular Biology Insights, Cascade, CO). These programs allow the user to input desired hybridization conditions such as melting temperature of a primer-template duplex for example. In some embodiments, an *in silico* PCR search algorithm, such as (ePCR) is used to analyze primer specificity across a plurality of template sequences which can be readily obtained from public sequence databases such as GenBank for example. An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety). This also provides information on primer specificity of

the selected primer pairs. In some embodiments, the hybridization conditions applied to the algorithm can limit the results of primer specificity obtained from the algorithm. In some embodiments, the melting temperature threshold for the primer template duplex is specified to be 35°C or a higher temperature. In some embodiments the number of acceptable mismatches is specified to be seven mismatches or less. In some embodiments, the buffer components and concentrations and primer concentrations may be specified and incorporated into the algorithm, for example, an appropriate primer concentration is about 250 nM and appropriate buffer components are 50 mM sodium or potassium and 1.5 mM Mg^{2+}.

[306] One with ordinary skill in the art of design of amplification primers will recognize that a given primer need not hybridize with 100% complementarity in order to effectively prime the synthesis of a complementary nucleic acid strand in an amplification reaction. Moreover, a primer may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event. (e.g., for example, a loop structure or a hairpin structure). The primers of the present invention may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity with any of the primers listed in Table 2. Thus, in some embodiments of the present invention, an extent of variation of 70% to 100%, or any range therewithin, of the sequence identity is possible relative to the specific primer sequences disclosed herein. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is identical to another 20 nucleobase primer having two non-identical residues has 18 of 20 identical residues (18/20 = 0.9 or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of primer 20 nucleobases in length would have 15/20 = 0.75 or 75% sequence identity with the 20 nucleobase primer.

[307] Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, complementarity of primers with respect to the conserved priming regions of viral nucleic acid is between about 70% and about 75% 80%. In other embodiments, homology, sequence identity or complementarity, is between about 75% and about 80%. In yet other embodiments, homology, sequence identity or complementarity, is at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is 100%.

[308] In some embodiments, the primers described herein comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%,

or at least 99%, or 100% (or any range therewithin) sequence identity with the primer sequences specifically disclosed herein.

[309] One with ordinary skill is able to calculate percent sequence identity or percent sequence homology and able to determine, without undue experimentation, the effects of variation of primer sequence identity on the function of the primer in its role in priming synthesis of a complementary strand of nucleic acid for production of an amplification product of a corresponding bioagent identifying amplicon.

[310] In one embodiment, the primers are at least 13 nucleobases in length. In another embodiment, the primers are less than 36 nucleobases in length.

[311] In some embodiments of the present invention, the oligonucleotide primers are 13 to 35 nucleobases in length (13 to 35 linked nucleotide residues). These embodiments comprise oligonucleotide primers 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleobases in length, or any range therewithin. The present invention contemplates using both longer and shorter primers. Furthermore, the primers may also be linked to one or more other desired moieties, including, but not limited to, affinity groups, ligands, regions of nucleic acid that are not complementary to the nucleic acid to be amplified, labels, etc. Primers may also form hairpin structures. For example, hairpin primers may be used to amplify short target nucleic acid molecules. The presence of the hairpin may stabilize the amplification complex (see e.g., TAQMAN MicroRNA Assays, Applied Biosystems, Foster City, California).

[312] In some embodiments, any oligonucleotide primer pair may have one or both primers with less then 70% sequence homology with a corresponding member of any of the primer pairs of Table 2 if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon. In other embodiments, any oligonucleotide primer pair may have one or both primers with a length greater than 35 nucleobases if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon.

[313] In some embodiments, the function of a given primer may be substituted by a combination of two or more primers segments that hybridize adjacent to each other or that are linked by a nucleic acid loop structure or linker which allows a polymerase to extend the two or more primers in an amplification reaction.

[314] In some embodiments, the primer pairs used for obtaining bioagent identifying amplicons are the primer pairs of Table 2. In other embodiments, other combinations of primer pairs are possible by combining certain members of the forward primers with certain members of the reverse primers. An example can be seen in Table 2 for two primer pair combinations of forward primer 16S_EC_789_810_F (SEQ ID NO: 206), with the reverse primers 16S_EC_880_894_R (SEQ ID NO: 796), or 16S_EC_882_899_R or (SEQ ID NO: 818). Arriving at a favorable alternate combination of primers in a primer pair depends upon the properties of the primer pair, most notably the size of the bioagent identifying amplicon that would be produced by the primer pair, which preferably is between about 45 to about 150 nucleobases in length. Alternatively, a bioagent identifying amplicon longer than 150 nucleobases in length could be cleaved into smaller segments by cleavage reagents such as chemical reagents, or restriction enzymes, for example.

[315] In some embodiments, the primers are configured to amplify nucleic acid of a bioagent to produce amplification products that can be measured by mass spectrometry and from whose molecular masses candidate base compositions can be readily calculated.

[316] In some embodiments, any given primer comprises a modification comprising the addition of a non-templated T residue to the 5' end of the primer (i.e., the added T residue does not necessarily hybridize to the nucleic acid being amplified). The addition of a non-templated T residue has an effect of minimizing the addition of non-templated adenosine residues as a result of the non-specific enzyme activity of *Taq* polymerase (Magnuson et al., Biotechniques, 1996, 21, 700-709), an occurrence which may lead to ambiguous results arising from molecular mass analysis.

[317] In some embodiments of the present invention, primers may contain one or more universal bases. Because any variation (due to codon wobble in the 3rd position) in the conserved regions among species is likely to occur in the third position of a DNA (or RNA) triplet, oligonucleotide primers can be designed such that the nucleotide corresponding to this position is a base which can bind to more than one nucleotide, referred to herein as a "universal nucleobase." For example, under this "wobble" pairing, inosine (I) binds to U, C or A; guanine (G) binds to U or C, and uridine (U) binds to U or C. Other examples of universal nucleobases include nitroindoles such as 5-nitroindole or 3-nitropyrrole (Loakes et al., Nucleosides and Nucleotides, 1995, 14, 1001-1003), the degenerate nucleotides **dP** or **dK** (Hill *et al.*), an acyclic nucleoside analog containing 5-nitroindazole (Van Aerschot et al., Nucleosides and Nucleotides, 1995, 14, 1053-1056) or the purine analog 1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (Sala et al., Nucl. Acids Res., 1996, 24, 3302-3306).

[318] In some embodiments, to compensate for the somewhat weaker binding by the wobble base, the oligonucleotide primers are designed such that the first and second positions of each triplet are occupied by nucleotide analogs that bind with greater affinity than the unmodified nucleotide. Examples of these analogs include, but are not limited to, 2,6-diaminopurine which binds to thymine, 5-propynyluracil (also known as propynylated thymine) which binds to adenine and 5-propynylcytosine and phenoxazines, including G-clamp, which binds to G. Propynylated pyrimidines are described in U.S. Patent Nos. 5,645,985, 5,830,653 and 5,484,908, each of which is commonly owned and incorporated herein by reference in its entirety. Propynylated primers are described in U.S Pre-Grant Publication No. 2003-0170682, which is also commonly owned and incorporated herein by reference in its entirety. Phenoxazines are described in U.S. Patent Nos. 5,502,177, 5,763,588, and 6,005,096, each of which is incorporated herein by reference in its entirety. G-clamps are described in U.S. Patent Nos. 6,007,992 and 6,028,183, each of which is incorporated herein by reference in its entirety.

[319] In some embodiments, primer hybridization is enhanced using primers containing 5-propynyl deoxy-cytidine and deoxy-thymidine nucleotides. These modified primers offer increased affinity and base pairing selectivity.

[320] In some embodiments, non-template primer tags are used to increase the melting temperature (T_m) of a primer-template duplex in order to improve amplification efficiency. A non-template tag is at least three consecutive A or T nucleotide residues on a primer which are not complementary to the template. In any given non-template tag, A can be replaced by C or G and T can also be replaced by C or G. Although Watson-Crick hybridization is not expected to occur for a non-template tag relative to the template, the extra hydrogen bond in a G-C pair relative to an A-T pair confers increased stability of the primer-template duplex and improves amplification efficiency for subsequent cycles of amplification when the primers hybridize to strands synthesized in previous cycles.

[321] In other embodiments, propynylated tags may be used in a manner similar to that of the non-template tag, wherein two or more 5-propynylcytidine or 5-propynyluridine residues replace template matching residues on a primer. In other embodiments, a primer contains a modified internucleoside linkage such as a phosphorothioate linkage, for example.

[322] In some embodiments, the primers contain mass-modifying tags. Reducing the total number of possible base compositions of a nucleic acid of specific molecular weight provides a means of avoiding a persistent source of ambiguity in determination of base composition of amplification products. Addition of mass-modifying tags to certain nucleobases of a given primer will result in simplification of *de novo* determination of base composition of a given bioagent identifying amplicon from its molecular mass.

[323] In some embodiments of the present invention, the mass modified nucleobase comprises one or more of the following: for example, 7-deaza-2'-deoxyadenosine-5-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxycytidine-5'-triphosphate, 5-iodo-2'-deoxycytidine-5'-triphosphate, 5-hydroxy-2'-deoxyuridine-5'-triphosphate, 4-thiothymidine-5'-triphosphate, 5-aza-2'-deoxyuridine-5'-triphosphate, 5-fluoro-2'-deoxyuridine-5'-triphosphate, O6-methyl-2'-deoxyguanosine-5'-triphosphate, N2-methyl-2'-deoxyguanosine-5'-triphosphate, 8-oxo-2'-deoxyguanosine-5'-triphosphate or thiothymidine-5'-triphosphate. In some embodiments, the mass-modified nucleobase comprises ^{15}N or ^{13}C or both ^{15}N and ^{13}C.

[324] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with a plurality of primer pairs. The advantages of multiplexing are that fewer reaction containers (for example, wells of a 96- or 384-well plate) are needed for each molecular mass measurement, providing time, resource and cost savings because additional bioagent identification data can be obtained within a single analysis. Multiplex amplification methods are well known to those with ordinary skill and can be developed without undue experimentation. However, in some embodiments, one useful and non-obvious step in selecting a plurality candidate bioagent identifying amplicons for multiplex amplification is to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results. In some embodiments, a 10 Da difference in mass of two strands of one or more amplification products is sufficient to avoid overlap of mass spectral peaks.

[325] In some embodiments, as an alternative to multiplex amplification, single amplification reactions can be pooled before analysis by mass spectrometry. In these embodiments, as for multiplex amplification embodiments, it is useful to select a plurality of candidate bioagent identifying amplicons to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results.

C Determination of Molecular Mass of Bioagent Identifying Amplicons

[326] In some embodiments, the molecular mass of a given bioagent identifying amplicon is determined by mass spectrometry. Mass spectrometry has several advantages, not the least of which is high bandwidth characterized by the ability to separate (and isolate) many molecular peaks across a broad range of mass to charge ratio (m/z). Thus mass spectrometry is intrinsically a parallel detection scheme without the need for radioactive or fluorescent labels, since every amplification product is identified by its molecular mass. The current state of the art in mass spectrometry is such that less than femtomole quantities of material can be readily analyzed to afford information about the molecular contents of the

sample. An accurate assessment of the molecular mass of the material can be quickly obtained, irrespective of whether the molecular weight of the sample is several hundred, or in excess of one hundred thousand atomic mass units (amu) or Daltons.

[327] In some embodiments, intact molecular ions are generated from amplification products using one of a variety of ionization techniques to convert the sample to gas phase. These ionization methods include, but are not limited to, electrospray ionization (ES), matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB). Upon ionization, several peaks are observed from one sample due to the formation of ions with different charges. Averaging the multiple readings of molecular mass obtained from a single mass spectrum affords an estimate of molecular mass of the bioagent identifying amplicon. Electrospray ionization mass spectrometry (ESI-MS) is particularly useful for very high molecular weight polymers such as proteins and nucleic acids having molecular weights greater than 10 kDa, since it yields a distribution of multiply-charged molecules of the sample without causing a significant amount of fragmentation.

[328] The mass detectors used in the methods of the present invention include, but are not limited to, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), time of flight (TOF), ion trap, quadrupole, magnetic sector, Q-TOF, and triple quadrupole.

D. Base Compositions of Bioagent Identifying Amplicons

[329] Although the molecular mass of amplification products obtained using intelligent primers provides a means for identification of bioagents, conversion of molecular mass data to a base composition signature is useful for certain analyses. As used herein, "base composition" is the exact number of each nucleobase (A, T, C and G) determined from the molecular mass of a bioagent identifying amplicon. In some embodiments, a base composition provides an index of a specific organism. Base compositions can be calculated from known sequences of known bioagent identifying amplicons and can be experimentally determined by measuring the molecular mass of a given bioagent identifying amplicon, followed by determination of all possible base compositions which are consistent with the measured molecular mass within acceptable experimental error. The following example illustrates determination of base composition from an experimentally obtained molecular mass of a 46-mer amplification product originating at position 1337 of the 16S rRNA of *Bacillus anthracis*. The forward and reverse strands of the amplification product have measured molecular masses of 14208 and 14079 Da, respectively. The possible base compositions derived from the molecular masses of the forward and reverse strands for the *B. anthracis* products are listed in Table 1.

Table 1

Possible Base Compositions for *B. anthracis* 46mer Amplification Product

Calc. Mass Forward Strand	Mass Error Forward Strand	Base Composition of Forward Strand	Calc. Mass Reverse Strand	Mass Error Reverse Strand	Base Composition of Reverse Strand
14208.2935	0.079520	A1 G17 C10 T18	14079.2624	0.080600	A0 G14 C13 T19
14208.3160	0.056980	A1 G20 C15 T10	14079.2849	0.058060	A0 G17 C18 T11
14208.3386	0.034440	A1 G23 C20 T2	14079.3075	0.035520	A0 G20 C23 T3
14208.3074	0.065560	A6 G11 C3 T26	14079.2538	0.089180	A5 G5 C1 T35
14208.3300	0.043020	A6 G14 C8 T18	14079.2764	0.066640	A5 G8 C6 T27
14208.3525	0.020480	A6 G17 C13 T10	14079.2989	0.044100	A5 G11 C11 T19
14208.3751	0.002060	A6 G20 C18 T2	14079.3214	0.021560	A5 G14 C16 T11
14208.3439	0.029060	A11 G8 C1 T26	14079.3440	0.000980	A5 G17 C21 T3
14208.3665	0.006520	A11 G11 C6 T18	14079.3129	0.030140	A10 G5 C4 T27
14208.3890	**0.016020**	**A11 G14 C11 T10**	14079.3354	0.007600	A10 G8 C9 T19
14208.4116	0.038560	A11 G17 C16 T2	**14079.3579**	**0.014940**	**A10 G11 C14 T11**
14208.4030	0.029980	A16 G8 C4 T18	14079.3805	0.037480	A10 G14 C19 T3
14208.4255	0.052520	A16 G11 C9 T10	14079.3494	0.006360	A15 G2 C2 T27
14208.4481	0.075060	A16 G14 C14 T2	14079.3719	0.028900	A15 G5 C7 T19
14208.4395	0.066480	A21 G5 C2 T18	14079.3944	0.051440	A15 G8 C12 T11
14208.4620	0.089020	A21 G8 C7 T10	14079.4170	0.073980	A15 G11 C17 T3
-	-	-	14079.4084	0.065400	A20 G2 C5 T19
-	-	-	14079.4309	0.087940	A20 G5 C10 T13

[330] Among the 16 possible base compositions for the forward strand and the 18 possible base compositions for the reverse strand that were calculated, only one pair (shown in **bold**) are complementary base compositions, which indicates the true base composition of the amplification product. It should be recognized that this logic is applicable for determination of base compositions of any bioagent identifying amplicon, regardless of the class of bioagent from which the corresponding amplification product was obtained.

[331] In some embodiments, assignment of previously unobserved base compositions (also known as "true unknown base compositions") to a given phylogeny can be accomplished via the use of pattern classifier model algorithms. Base compositions, like sequences, vary slightly from strain to strain within species, for example. In some embodiments, the pattern classifier model is the mutational probability model. On other embodiments, the pattern classifier is the polytope model. The mutational probability model and polytope model are both commonly owned and described in U.S. Patent application Serial No. 11/073,362 which is incorporated herein by reference in entirety.

[332] In one embodiment, it is possible to manage this diversity by building "base composition probability clouds" around the composition constraints for each species. This permits identification of

organisms in a fashion similar to sequence analysis. A "pseudo four-dimensional plot" can be used to visualize the concept of base composition probability clouds. Optimal primer design requires optimal choice of bioagent identifying amplicons and maximizes the separation between the base composition signatures of individual bioagents. Areas where clouds overlap indicate regions that may result in a misclassification, a problem which is overcome by a triangulation identification process using bioagent identifying amplicons not affected by overlap of base composition probability clouds.

[333] In some embodiments, base composition probability clouds provide the means for screening potential primer pairs in order to avoid potential misclassifications of base compositions. In other embodiments, base composition probability clouds provide the means for predicting the identity of a bioagent whose assigned base composition was not previously observed and/or indexed in a bioagent identifying amplicon base composition database due to evolutionary transitions in its nucleic acid sequence. Thus, in contrast to probe-based techniques, mass spectrometry determination of base composition does not require prior knowledge of the composition or sequence in order to make the measurement.

[334] The present invention provides bioagent classifying information similar to DNA sequencing and phylogenetic analysis at a level sufficient to identify a given bioagent. Furthermore, the process of determination of a previously unknown base composition for a given bioagent (for example, in a case where sequence information is unavailable) has downstream utility by providing additional bioagent indexing information with which to populate base composition databases. The process of future bioagent identification is thus greatly improved as more BCS indexes become available in base composition databases.

E. Triangulation Identification

[335] In some cases, a molecular mass of a single bioagent identifying amplicon alone does not provide enough resolution to unambiguously identify a given bioagent. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by determining the molecular masses of a plurality of bioagent identifying amplicons selected within a plurality of housekeeping genes. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[336] In some embodiments, the triangulation identification process can be pursued by characterization of bioagent identifying amplicons in a massively parallel fashion using the polymerase chain reaction (PCR), such as multiplex PCR where multiple primers are employed in the same amplification reaction mixture, or PCR in multi-well plate format wherein a different and unique pair of primers is used in multiple wells containing otherwise identical reaction mixtures. Such multiplex and multi-well PCR methods are well known to those with ordinary skill in the arts of rapid throughput amplification of nucleic acids. In other related embodiments, one PCR reaction per well or container may be carried out, followed by an amplicon pooling step wherein the amplification products of different wells are combined in a single well or container which is then subjected to molecular mass analysis. The combination of pooled amplicons can be chosen such that the expected ranges of molecular masses of individual amplicons are not overlapping and thus will not complicate identification of signals.

F. Codon Base Composition Analysis

[337] In some embodiments of the present invention, one or more nucleotide substitutions within a codon of a gene of an infectious organism confer drug resistance upon an organism which can be determined by codon base composition analysis. The organism can be a bacterium, virus, fungus or protozoan.

[338] In some embodiments, the amplification product containing the codon being analyzed is of a length of about 35 to about 200 nucleobases. The primers employed in obtaining the amplification product can hybridize to upstream and downstream sequences directly adjacent to the codon, or can hybridize to upstream and downstream sequences one or more sequence positions away from the codon. The primers may have between about 70% to 100% sequence complementarity with the sequence of the gene containing the codon being analyzed.

[339] In some embodiments, the codon base composition analysis is undertaken

[340] In some embodiments, the codon analysis is undertaken for the purpose of investigating genetic disease in an individual. In other embodiments, the codon analysis is undertaken for the purpose of investigating a drug resistance mutation or any other deleterious mutation in an infectious organism such as a bacterium, virus, fungus or protozoan. In some embodiments, the bioagent is a bacterium identified in a biological product.

[341] In some embodiments, the molecular mass of an amplification product containing the codon being analyzed is measured by mass spectrometry. The mass spectrometry can be either electrospray (ESI) mass spectrometry or matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

Time-of-flight (TOF) is an example of one mode of mass spectrometry compatible with the analyses of the present invention.

[342] The methods of the present invention can also be employed to determine the relative abundance of drug resistant strains of the organism being analyzed. Relative abundances can be calculated from amplitudes of mass spectral signals with relation to internal calibrants. In some embodiments, known quantities of internal amplification calibrants can be included in the amplification reactions and abundances of analyte amplification product estimated in relation to the known quantities of the calibrants.

[343] In some embodiments, upon identification of one or more drug-resistant strains of an infectious organism infecting an individual, one or more alternative treatments can be devised to treat the individual.

G. Determination of the Quantity of a Bioagent

[344] In some embodiments, the identity and quantity of an unknown bioagent can be determined using the process illustrated in Figure 2. Primers (**500**) and a known quantity of a calibration polynucleotide (**505**) are added to a sample containing nucleic acid of an unknown bioagent. The total nucleic acid in the sample is then subjected to an amplification reaction (**510**) to obtain amplification products. The molecular masses of amplification products are determined (**515**) from which are obtained molecular mass and abundance data. The molecular mass of the bioagent identifying amplicon (**520**) provides the means for its identification (**525**) and the molecular mass of the calibration amplicon obtained from the calibration polynucleotide (**530**) provides the means for its identification (**535**). The abundance data of the bioagent identifying amplicon is recorded (**540**) and the abundance data for the calibration data is recorded (**545**), both of which are used in a calculation (**550**) which determines the quantity of unknown bioagent in the sample.

[345] A sample comprising an unknown bioagent is contacted with a pair of primers that provide the means for amplification of nucleic acid from the bioagent, and a known quantity of a polynucleotide that comprises a calibration sequence. The nucleic acids of the bioagent and of the calibration sequence are amplified and the rate of amplification is reasonably assumed to be similar for the nucleic acid of the bioagent and of the calibration sequence. The amplification reaction then produces two amplification products: a bioagent identifying amplicon and a calibration amplicon. The bioagent identifying amplicon and the calibration amplicon should be distinguishable by molecular mass while being amplified at essentially the same rate. Effecting differential molecular masses can be accomplished by choosing as a calibration sequence, a representative bioagent identifying amplicon (from a specific species of bioagent) and performing, for example, a 2-8 nucleobase deletion or insertion within the variable region between

the two priming sites. The amplified sample containing the bioagent identifying amplicon and the calibration amplicon is then subjected to molecular mass analysis by mass spectrometry, for example. The resulting molecular mass analysis of the nucleic acid of the bioagent and of the calibration sequence provides molecular mass data and abundance data for the nucleic acid of the bioagent and of the calibration sequence. The molecular mass data obtained for the nucleic acid of the bioagent enables identification of the unknown bioagent and the abundance data enables calculation of the quantity of the bioagent, based on the knowledge of the quantity of calibration polynucleotide contacted with the sample.

[346] In some embodiments, construction of a standard curve where the amount of calibration polynucleotide spiked into the sample is varied provides additional resolution and improved confidence for the determination of the quantity of bioagent in the sample. The use of standard curves for analytical determination of molecular quantities is well known to one with ordinary skill and can be performed without undue experimentation.

[347] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with multiple primer pairs which also amplify the corresponding standard calibration sequences. In this or other embodiments, the standard calibration sequences are optionally included within a single vector which functions as the calibration polynucleotide. Multiplex amplification methods are well known to those with ordinary skill and can be performed without undue experimentation.

[348] In some embodiments, the calibrant polynucleotide is used as an internal positive control to confirm that amplification conditions and subsequent analysis steps are successful in producing a measurable amplicon. Even in the absence of copies of the genome of a bioagent, the calibration polynucleotide should give rise to a calibration amplicon. Failure to produce a measurable calibration amplicon indicates a failure of amplification or subsequent analysis step such as amplicon purification or molecular mass determination. Reaching a conclusion that such failures have occurred is in itself, a useful event.

[349] In some embodiments, the calibration sequence is comprised of DNA. In some embodiments, the calibration sequence is comprised of RNA.

[350] In some embodiments, the calibration sequence is inserted into a vector that itself functions as the calibration polynucleotide. In some embodiments, more than one calibration sequence is inserted into the vector that functions as the calibration polynucleotide. Such a calibration polynucleotide is herein termed a "combination calibration polynucleotide." The process of inserting polynucleotides into vectors

is routine to those skilled in the art and can be accomplished without undue experimentation. Thus, it should be recognized that the calibration method should not be limited to the embodiments described herein. The calibration method can be applied for determination of the quantity of any bioagent identifying amplicon when an appropriate standard calibrant polynucleotide sequence is designed and used. The process of choosing an appropriate vector for insertion of a calibrant is also a routine operation that can be accomplished by one with ordinary skill without undue experimentation.

H. Identification of Bacteria

[351] In other embodiments of the present invention, the primer pairs produce bioagent identifying amplicons within stable and highly conserved regions of bacteria. The advantage to characterization of an amplicon defined by priming regions that fall within a highly conserved region is that there is a low probability that the region will evolve past the point of primer recognition, in which case, the primer hybridization of the amplification step would fail. Such a primer set is thus useful as a broad range survey-type primer. In another embodiment of the present invention, the intelligent primers produce bioagent identifying amplicons including a region which evolves more quickly than the stable region described above. The advantage of characterization bioagent identifying amplicon corresponding to an evolving genomic region is that it is useful for distinguishing emerging strain variants or the presence of virulence genes, drug resistance genes, or codon mutations that induce drug resistance.

[352] The present invention also has significant advantages as a platform for identification of diseases caused by emerging bacterial strains such as, for example, drug-resistant strains of *Staphylococcus aureus*. The present invention eliminates the need for prior knowledge of bioagent sequence to generate hybridization probes. This is possible because the methods are not confounded by naturally occurring evolutionary variations occurring in the sequence acting as the template for production of the bioagent identifying amplicon. Measurement of molecular mass and determination of base composition is accomplished in an unbiased manner without sequence prejudice.

[353] Another embodiment of the present invention also provides a means of tracking the spread of a bacterium, such as a particular drug-resistant strain when a plurality of samples obtained from different locations are analyzed by the methods described above in an epidemiological setting. In one embodiment, a plurality of samples from a plurality of different locations is analyzed with primer pairs which produce bioagent identifying amplicons, a subset of which contains a specific drug-resistant bacterial strain. The corresponding locations of the members of the drug-resistant strain subset indicate the spread of the specific drug-resistant strain to the corresponding locations.

I. Kits

[354] The present invention also provides kits for carrying out the methods described herein. In some embodiments, the kit may comprise a sufficient quantity of one or more primer pairs to perform an amplification reaction on a target polynucleotide from a bioagent to form a bioagent identifying amplicon. In some embodiments, the kit may comprise from one to fifty primer pairs, from one to twenty primer pairs, from one to ten primer pairs, or from two to five primer pairs. In some embodiments, the kit may comprise one or more primer pairs recited in Table 2.

[355] In some embodiments, the kit comprises one or more broad range survey primer(s), division wide primer(s), or drill-down primer(s), or any combination thereof. If a given problem involves identification of a specific bioagent, the solution to the problem may require the selection of a particular combination of primers to provide the solution to the problem. A kit may be designed so as to comprise particular primer pairs for identification of a particular bioagent. A drill-down kit may be used, for example, to distinguish different genotypes or strains, drug-resistant, or otherwise. In some embodiments, the primer pair components of any of these kits may be additionally combined to comprise additional combinations of broad range survey primers and division-wide primers so as to be able to identify a bacterium.

[356] In some embodiments, the kit contains standardized calibration polynucleotides for use as internal amplification calibrants. Internal calibrants are described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[357] In some embodiments, the kit comprises a sufficient quantity of reverse transcriptase (if RNA is to be analyzed for example), a DNA polymerase, suitable nucleoside triphosphates (including alternative dNTPs such as inosine or modified dNTPs such as the 5-propynyl pyrimidines or any dNTP containing molecular mass-modifying tags such as those described above), a DNA ligase, and/or reaction buffer, or any combination thereof, for the amplification processes described above. A kit may further include instructions pertinent for the particular embodiment of the kit, such instructions describing the primer pairs and amplification conditions for operation of the method. A kit may also comprise amplification reaction containers such as microcentrifuge tubes and the like. A kit may also comprise reagents or other materials for isolating bioagent nucleic acid or bioagent identifying amplicons from amplification, including, for example, detergents, solvents, or ion exchange resins which may be linked to magnetic beads. A kit may also comprise a table of measured or calculated molecular masses and/or base compositions of bioagents using the primer pairs of the kit.

[358] Some embodiments are kits that contain one or more survey bacterial primer pairs represented by primer pair compositions wherein each member of each pair of primers has 70% to 100% sequence

identity with the corresponding member from the group of primer pairs represented by any of the primer pairs of Table 5. The survey primer pairs may include broad range primer pairs which hybridize to ribosomal RNA, and may also include division-wide primer pairs which hybridize to housekeeping genes such as rplB, tufB, rpoB, rpoC, valS, and infB, for example.

[359] In some embodiments, a kit may contain one or more survey bacterial primer pairs and one or more triangulation genotyping analysis primer pairs such as the primer pairs of Tables 8, 12, 14, 19, 21, 23, or 24. In some embodiments, the kit may represent a less expansive genotyping analysis but include triangulation genotyping analysis primer pairs for more than one genus or species of bacteria. For example, a kit for surveying nosocomial infections at a health care facility may include, for example, one or more broad range survey primer pairs, one or more division wide primer pairs, one or more *Acinetobacter baumannii* triangulation genotyping analysis primer pairs and one or more *Staphylococcus aureus* triangulation genotyping analysis primer pairs. One with ordinary skill will be capable of analyzing *in silico* amplification data to determine which primer pairs will be able to provide optimal identification resolution for the bacterial bioagents of interest.

[360] In some embodiments, a kit may be assembled for identification of strains of bacteria involved in contamination of food. An example of such a kit embodiment is a kit comprising one or more bacterial survey primer pairs of Table 5 with one or more triangulation genotyping analysis primer pairs of Table 12 which provide strain resolving capabilities for identification of specific strains of *Campylobacter jejuni*.

[361] Some embodiments of the kits are 96-well or 384-well plates with a plurality of wells containing any or all of the following components: dNTPs, buffer salts, Mg^{2+}, betaine, and primer pairs. In some embodiments, a polymerase is also included in the plurality of wells of the 96-well or 384-well plates.

[362] Some embodiments of the kit contain instructions for PCR and mass spectrometry analysis of amplification products obtained using the primer pairs of the kits.

[363] Some embodiments of the kit include a barcode which uniquely identifies the kit and the components contained therein according to production lots and may also include any other information relative to the components such as concentrations, storage temperatures, etc. The barcode may also include analysis information to be read by optical barcode readers and sent to a computer controlling amplification, purification and mass spectrometric measurements. In some embodiments, the barcode provides access to a subset of base compositions in a base composition database which is in digital

communication with base composition analysis software such that a base composition measured with primer pairs from a given kit can be compared with known base compositions of bioagent identifying amplicons defined by the primer pairs of that kit.

[364] In some embodiments, the kit contains a database of base compositions of bioagent identifying amplicons defined by the primer pairs of the kit. The database is stored on a convenient computer readable medium such as a compact disk or USB drive, for example.

[365] In some embodiments, the kit includes a computer program stored on a computer formatted medium (such as a compact disk or portable USB disk drive, for example) comprising instructions which direct a processor to analyze data obtained from the use of the primer pairs of the present invention. The instructions of the software transform data related to amplification products into a molecular mass or base composition which is a useful concrete and tangible result used in identification and/or classification of bioagents. In some embodiments, the kits of the present invention contain all of the reagents sufficient to carry out one or more of the methods described herein.

[366] While the present invention has been described with specificity in accordance with certain of its embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

EXAMPLES

Example 1: Design and Validation of Primers that Define Bioagent Identifying Amplicons for Identification of Bacteria

[367] For design of primers that define bacterial bioagent identifying amplicons, a series of bacterial genome segment sequences were obtained, aligned and scanned for regions where pairs of PCR primers would amplify products of about 45 to about 150 nucleotides in length and distinguish subgroups and/or individual strains from each other by their molecular masses or base compositions. A typical process shown in Figure 1 is employed for this type of analysis.

[368] A database of expected base compositions for each primer region was generated using an *in silico* PCR search algorithm, such as (ePCR). An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated

herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs.

[369] Table 2 represents a collection of primers (sorted by primer pair number) designed to identify bacteria using the methods described herein. The primer pair number is an in-house database index number. Primer sites were identified on segments of genes, such as, for example, the 16S rRNA gene. The forward or reverse primer name shown in Table 2 indicates the gene region of the bacterial genome to which the primer hybridizes relative to a reference sequence. In Table 2, for example, the forward primer name `16S_EC_1077_1106_F` indicates that the forward primer (`_F`) hybridizes to residues 1077-1106 of the reference sequence represented by a sequence extraction of coordinates 4033120..4034661 from GenBank gi number 16127994 (as indicated in Table 3). As an additional example: the forward primer name BONTA_X52066_450_473 indicates that the primer hybridizes to residues 450-437 of the gene encoding *Clostridium botulinum* neurotoxin type A (BoNT/A) represented by GenBank Accession No. X52066 (primer pair name codes appearing in Table 2 are defined in Table 3. One with ordinary skill knows how to obtain individual gene sequences or portions thereof from genomic sequences present in GenBank. In Table 2, Tp = 5-propynyluracil; Cp = 5-propynylcytosine; * = phosphorothioate linkage; I = inosine. T. GenBank Accession Numbers for reference sequences of bacteria are shown in Table 3 (below). In some cases, the reference sequences are extractions from bacterial genomic sequences or complements thereof.

Table 2: Primer Pairs for Identification of Bacteria

Primer Pair Number	Forward Primer Name	Forward Sequence	Forward SEQ ID NO:	Reverse Primer Name	Reverse Sequence	Reverse SEQ ID NO:
1	16S_EC_1077_1106_F	GTGAGATGTTGGGTTAAGTCCCGTAACGAG	134	16S_EC_1175_1195_R	GACGTCATCCCCACCTTCCTC	809
2	16S_EC_1082_1106_F	ATGTTGGGTTAAGTCCCGCAACGAG	38	16S_EC_1175_1197_R	TTGACGTCATCCCCACCTTCCTC	1398
3	16S_EC_1090_1111_F	TTAAGTCCCGCAACGATCGCAA	651	16S_EC_1175_1196_R	TGACGTCATCCCCACCTTCCTC	1159
4	16S_EC_1222_1241_F	GCTACACACGTGCTACAATG	114	16S_EC_1303_1323_R	CGAGTTGCAGACTGCGATCCG	787
5	16S_EC_1332_1353_F	AAGTCGGAATCGCTAGTAATCG	10	16S_EC_1389_1407_R	GACGGGCGGTGTGTACAAG	806
6	16S_EC_30_54_F	TGAACGCTGGTGGCATGCTTAACAC	429	16S_EC_105_126_R	TACGCATTACTCACCCGTCCGC	897
7	16S_EC_38_64_F	GTGGCATGCCTAATACATGCAAGTCG	136	16S_EC_101_120_R	TTACTCACCCGTCCGCCGCT	1365
8	16S_EC_49_68_F	TAACACATGCAAGTCGAACG	152	16S_EC_104_120_R	TTACTCACCCGTCCGCC	1364
9	16S_EC_683_700_F	GTGTAGCGGTGAAATGCG	137	16S_EC_774_795_R	GTATCTAATCCTGTTTGCTCCC	839
10	16S_EC_713_732_F	AGAACACCGATGGCGAAGGC	21	16S_EC_789_809_R	CGTGGACTACCAGGGTATCTA	798
11	16S_EC_785_806_F	GGATTAGAGACCCTGGTAGTCC	118	16S_EC_880_897_R	GGCCGTACTCCCCAGGCG	830
12	16S_EC_785_810_F	GGATTAGATACCCTGGTAGTCCACGC	119	16S_EC_880_897_2_R	GGCCGTACTCCCCAGGCG	830
13	16S_EC_789_810_F	TAGATACCCTGGTAGTCCACGC	206	16S_EC_880_894_R	CGTACTCCCCAGGCG	796
14	16S_EC_960_981_F	TTCGATGCAACGCGAAGAACCT	672	16S_EC_1054_1073_R	ACGAGCTGACGACAGCCATG	735
15	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078_R	ACGACACGAGCTGACGAC	734
16	23S_EC_1826_1843_F	CTGACACCTGCCCGGTGC	80	23S_EC_1906_1924_R	GACCGTTATAGTTACGGCC	805
17	23S_EC_2645_2669_F	TCTGTCCCTAGTACGAGAGGACCGG	408	23S_EC_2744_2761_R	TGCTTAGATGCTTTCAGC	1252
18	23S_EC_2645_2669_2_F	CTGTCCCTAGTACGAGAGGACCGG	83	23S_EC_2751_2767_R	GTTTCATGCTTAGATGCTTTCAGC	846
19	23S_EC_493_518_F	GGGGAGTGAAAGAGATCCTGAAACCG	125	23S_EC_551_571_R	ACAAAAGGTACGCCGTCACCC	717
20	23S_EC_493_518_2_F	GGGGAGTGAAAGAGATCCTGAAACCG	125	23S_EC_551_571_2_R	ACAAAAGGCACGCCATCACCC	716
21	23S_EC_971_992_F	CGAGAGGGAAACAACCCAGACC	66	23S_EC_1059_1077_R	TGGCTGCTTCTAAGCCAAC	1282
22	CAPC_BA_104_131_F	GTTATTTAGCACTCGTTTTTAATCAGCC	139	CAPC_BA_180_205_R	TGAATCTTGAAACACCATACGTAACG	1150
23	CAPC_BA_114_133_F	ACTCGTTTTTAATCAGCCCG	20	CAPC_BA_185_205_R	TGAATCTTGAAACACCATACG	1149
24	CAPC_BA_274_303_F	GATTATTGTTATCCTGTTATGCCATTTGAG	109	CAPC_BA_349_376_R	GTAACCCTTGTCTTTGAATTGTATTTGC	837
25	CAPC_BA_276_296_F	TTATTGTTATCCTGTTATGCC	663	CAPC_BA_358_377_R	GGTAACCCTTGTCTTTGAAT	834
26	CAPC_BA_281_301_F	GTTATCCTGTTATGCCATTTG	138	CAPC_BA_361_378_R	TGGTAACCCTTGTCTTTG	1298
27	CAPC_BA_315_334_F	CCGTGGTATTGGAGTTATTG	59	CAPC_BA_361_378_R	TGGTAACCCTTGTCTTTG	1298
28	CYA_BA_1055_1072_F	GAAAGAGTTCGGATTGGG	92	CYA_BA_1112_1130_R	TGTTGACCATGCTTCTTAG	1352

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29	CYA_BA_1349_1370_F	ACAACGAAGTACAATACAAGAC	12	CYA_BA_1447_1426_R	CTTCTACATTTTTAGCCATCAC	800
30	CYA_BA_1353_1379_F	CGAAGTACAATACAAGACAAAAGAAG G	64	CYA_BA_1448_1467_R	TGTTAACGGCTTCAAGACCC	1342
31	CYA_BA_1359_1379_F	ACAATACAAGACAAAAGAAGG	13	CYA_BA_1447_1461_R	CGGCTTCAAGACCCC	794
32	CYA_BA_914_937_F	CAGGTTTAGTACCAGAACATGCAG	53	CYA_BA_999_1026_R	ACCACTTTTAATAAGGTTTGTAGCTAAC	728
33	CYA_BA_916_935_F	GGTTTAGTACCAGAACATGC	131	CYA_BA_1003_1025_R	CCACTTTTAATAAGGTTTGTAGC	768
34	INFB_EC_1365_1393_F	TGCTCGTGGTGCACAAGTAACGGATA TTA	524	INFB_EC_1439_1467_R	TGCTGCTTTCGCATGGTTAATTGCTTCA A	1248
35	LEF_BA_1033_1052_F	TCAAGAAGAAAAAGAGC	254	LEF_BA_1119_1135_R	GAATATCAATTTGTAGC	803
36	LEF_BA_1036_1066_F	CAAGAAGAAAAAGAGCTTCTAAAAAG AATAC	44	LEF_BA_1119_1149_R	AGATAAAGAATCACGAATATCAATTTGT AGC	745
37	LEF_BA_756_781_F	AGCTTTTGCATATTATATCGAGCCAC	26	LEF_BA_843_872_R	TCTTCCAAGGATAGATTTATTTCTTGTT CG	1135
38	LEF_BA_758_778_F	CTTTTGCATATTATATCGAGC	90	LEF_BA_843_865_R	AGGATAGATTTATTTCTTGTTCG	748
39	LEF_BA_795_813_F	TTTACAGCTTTATGCACCG	700	LEF_BA_883_900_R	TCTTGACAGCATCCGTTG	1140
40	LEF_BA_883_899_F	CAACGGATGCTGGCAAG	43	LEF_BA_939_958_R	CAGATAAAGAATCGCTCCAG	762
41	PAG_BA_122_142_F	CAGAATCAAGTTCCCAGGGG	49	PAG_BA_190_209_R	CCTGTAGTAGAAGAGGTAAC	781
42	PAG_BA_123_145_F	AGAATCAAGTTCCCAGGGGTTAC	22	PAG_BA_187_210_R	CCCTGTAGTAGAAGAGGTAACCAC	774
43	PAG_BA_269_287_F	AATCTGCTATTTGGTCAGG	11	PAG_BA_326_344_R	TGATTATCAGCGGAAGTAG	1186
44	PAG_BA_655_675_F	GAAGGATATACGGTTGATGTC	93	PAG_BA_755_772_R	CCGTGCTCCATTTTTCAG	778
45	PAG_BA_753_772_F	TCCTGAAAAATGGAGCACGG	341	PAG_BA_849_868_R	TCGGATAAGCTGCCACAAGG	1089
46	PAG_BA_763_781_F	TGGAGCACGGCTTCTGATC	552	PAG_BA_849_868_R	TCGGATAAGCTGCCACAAGG	1089
47	RPOC_EC_1018_1045_F	CAAAACTTATTAGGTAAGCGTGTTGA CT	39	RPOC_EC_1095_1124_R	TCAAGCGCCATTTCTTTTGGTAAACCAC AT	959
48	RPOC_EC_1018_1045_2_F	CAAAACTTATTAGGTAAGCGTGTTGA CT	39	RPOC_EC_1095_1124_2_R	TCAAGCGCCATCTCTTTCGGTAATCCAC AT	958
49	RPOC_EC_114_140_F	TAAGAAGCCGGAAACCATCAACTACC G	158	RPOC_EC_213_232_R	GGCGCTTGTACTTACCGCAC	831
50	RPOC_EC_2178_2196_F	TGATTCTGGTGCCCGTGGT	478	RPOC_EC_2225_2246_R	TTGGCCATCAGGCCACGCATAC	1414
51	RPOC_EC_2178_2196_2_F	TGATTCCGGTGCCCGTGGT	477	RPOC_EC_2225_2246_2_R	TTGGCCATCAGACCACGCATAC	1413
52	RPOC_EC_2218_2241_F	CTGGCAGGTATGCGTGGTCTGATG	81	RPOC_EC_2313_2337_R	CGCACCGTGGGTTGAGATGAAGTAC	790
53	RPOC_EC_2218_2241_2_F	CTTGCTGGTATGCGTGGTCTGATG	86	RPOC_EC_2313_2337_2_R	CGCACCATGCGTAGAGATGAAGTAC	789
54	RPOC_EC_808_833_F	CGTCGGGTGATTAACCGTAACAACCG	75	RPOC_EC_865_889_R	GTTTTTCGTTGCGTACGATGATGTC	847
55	RPOC_EC_808_833_2_F	CGTCGTGTAATTAACCGTAACAACCG	76	RPOC_EC_865_891_R	ACGTTTTTCGTTTTGAACGATAATGCT	741
56	RPOC_EC_993_1019_F	CAAAGGTAAGCAAGGTCGTTTCCGTC A	41	RPOC_EC_1036_1059_R	CGAACGGCCTGAGTAGTCAACACG	785
57	RPOC_EC_993_1019_2_F	CAAAGGTAAGCAAGGACGTTTCCGTC A	40	RPOC_EC_1036_1059_2_R	CGAACGGCCAGAGTAGTCAACACG	784

58	SSPE_BA_115_137_F	CAAGCAAACGCACAATCAGAAGC	45	SSPE_BA_197_222_R	TGCACGTCTGTTTCAGTTGCAAATTC	1201
59	TUFB_EC_239_259_F	TAGACTGCCCAGGACACGCTG	204	TUFB_EC_283_303_R	GCCGTCCATCTGAGCAGCACC	815
60	TUFB_EC_239_259_2_F	TTGACTGCCCAGGTCACGCTG	678	TUFB_EC_283_303_2_R	GCCGTCCATTTGAGCAGCACC	816
61	TUFB_EC_976_1000_F	AACTACCGTCCGCAGTTCTACTTCC	4	TUFB_EC_1045_1068_R	GTTGTCGCCAGGCATAACCATTTC	845
62	TUFB_EC_976_1000_2_F	AACTACCGTCCTCAGTTCTACTTCC	5	TUFB_EC_1045_1068_2_R	GTTGTCACCAGGCATTACCATTTC	844
63	TUFB_EC_985_1012_F	CCACAGTTCTACTTCCGTACTACTGACG	56	TUFB_EC_1033_1062_R	TCCAGGCATTACCATTTCTACTCCTTCTGG	1006
66	RPLB_EC_650_679_F	GACCTACAGTAAGAGGTTCTGTAATGAACC	98	RPLB_EC_739_762_R	TCCAAGTGCTGGTTTACCCCATGG	999
67	RPLB_EC_688_710_F	CATCCACACGGTGGTGGTGAAGG	54	RPLB_EC_736_757_R	GTGCTGGTTTACCCCATGGAGT	842
68	RPOC_EC_1036_1060_F	CGTGTTGACTATTCGGGGCGTTCAG	78	RPOC_EC_1097_1126_R	ATTCAAGAGCCATTTCTTTTGGTAAACCAC	754
69	RPOB_EC_3762_3790_F	TCAACAACCTCTTGGAGGTAAAGCTCAGT	248	RPOB_EC_3836_3865_R	TTTCTTGAAGAGTATGAGCTGCTCCGTAAG	1435
70	RPLB_EC_688_710_F	CATCCACACGGTGGTGGTGAAGG	54	RPLB_EC_743_771_R	TGTTTTGTATCCAAGTGCTGGTTTACCCC	1356
71	VALS_EC_1105_1124_F	CGTGGCGGCGTGGTTATCGA	77	VALS_EC_1195_1218_R	CGGTACGAACTGGATGTCGCCGTT	795
72	RPOB_EC_1845_1866_F	TATCGCTCAGGCGAACTCCAAC	233	RPOB_EC_1909_1929_R	GCTGGATTCGCCTTTGCTACG	825
73	RPLB_EC_669_698_F	TGTAATGAACCCTAATGACCATCCACACGG	623	RPLB_EC_735_761_R	CCAAGTGCTGGTTTACCCCATGGAGTA	767
74	RPLB_EC_671_700_F	TAATGAACCCTAATGACCATCCACACGGTG	169	RPLB_EC_737_762_R	TCCAAGTGCTGGTTTACCCCATGGAG	1000
75	SP101_SPET11_1_29_F	AACCTTAATTGGAAAGAAACCCAAGAAGT	2	SP101_SPET11_92_116_R	CCTACCCAACGTTCACCAAGGGCAG	779
76	SP101_SPET11_118_147_F	GCTGGTGAAAATAACCCAGATGTCGTCTTC	115	SP101_SPET11_213_238_R	TGTGGCCGATTTCACCACCTGCTCCT	1340
77	SP101_SPET11_216_243_F	AGCAGGTGGTGAAATCGGCCACATGATT	24	SP101_SPET11_308_333_R	TGCCACTTTGACAACTCCTGTTGCTG	1209
78	SP101_SPET11_266_295_F	CTTGTACTTGTGGCTCACACGGCTGTTTGG	89	SP101_SPET11_355_380_R	GCTGCTTTGATGGCTGAATCCCCTTC	824
79	SP101_SPET11_322_344_F	GTCAAAGTGGCACGTTTACTGGC	132	SP101_SPET11_423_441_R	ATCCCCTGCTTCTGCTGCC	753
80	SP101_SPET11_358_387_F	GGGGATTCAGCCATCAAAGCAGCTATTGAC	126	SP101_SPET11_448_473_R	CCAACCTTTTCCACAACAGAATCAGC	766
81	SP101_SPET11_600_629_F	CCTTACTTCGAACTATGAATCTTTTGGAAG	62	SP101_SPET11_686_714_R	CCCATTTTTTCACGCATGCTGAAAATATC	772
82	SP101_SPET11_658_684_F	GGGGATTGATATCACCGATAAGAAGAA	127	SP101_SPET11_756_784_R	GATTGGCGATAAAGTGATATTTTCTAAAA	813
83	SP101_SPET11_776_801_F	TCGCCAATCAAAACTAAGGGAATGGC	364	SP101_SPET11_871_896_R	GCCCACCAGAAAGACTAGCAGGATAA	814
84	SP101_SPET11_893_921_F	GGGCAACAGCAGCGGATTGCGATTGCGCG	123	SP101_SPET11_988_1012_R	CATGACAGCCAAGACCTCACCCACC	763

85	SP101_SPET11_1154_1179_F	CAATACCGCAACAGCGGTGGCTTGGG	47	SP101_SPET11_1251_1277_R	GACCCCAACCTGGCCTTTTGTCGTTGA	804
86	SP101_SPET11_1314_1336_F	CGCAAAAAAATCCAGCTATTAGC	68	SP101_SPET11_1403_1431_R	AAACTATTTTTTTAGCTATACTCGAACAC	711
87	SP101_SPET11_1408_1437_F	CGAGTATAGCTAAAAAAATAGTTTATGACA	67	SP101_SPET11_1486_1515_R	GGATAATTGGTCGTAACAAGGGATAGTGAG	828
88	SP101_SPET11_1688_1716_F	CCTATATTAATCGTTTACAGAAACTGGCT	60	SP101_SPET11_1783_1808_R	ATATGATTATCATTGAACTGCGGCCG	752
89	SP101_SPET11_1711_1733_F	CTGGCTAAAACTTTGGCAACGGT	82	SP101_SPET11_1808_1835_R	GCGTGACGACCTTCTTGAATTGTAATCA	821
90	SP101_SPET11_1807_1835_F	ATGATTACAATTCAAGAAGGTCGTCACGC	33	SP101_SPET11_1901_1927_R	TTGGACCTGTAATCAGCTGAATACTGG	1412
91	SP101_SPET11_1967_1991_F	TAACGGTTATCATGGCCCAGATGGG	155	SP101_SPET11_2062_2083_R	ATTGCCCAGAAATCAAATCATC	755
92	SP101_SPET11_2260_2283_F	CAGAGACCGTTTTATCCTATCAGC	50	SP101_SPET11_2375_2397_R	TCTGGGTGACCTGGTGTTTTAGA	1131
93	SP101_SPET11_2375_2399_F	TCTAAAACACCAGGTCACCCAGAAG	390	SP101_SPET11_2470_2497_R	AGCTGCTAGATGAGCTTCTGCCATGGCC	747
94	SP101_SPET11_2468_2487_F	ATGGCCATGGCAGAAGCTCA	35	SP101_SPET11_2543_2570_R	CCATAAGGTCACCGTCACCATTCAAAGC	770
95	SP101_SPET11_2961_2984_F	ACCATGACAGAAGGCATTTTGACA	15	SP101_SPET11_3023_3045_R	GGAATTTACCAGCGATAGACACC	827
96	SP101_SPET11_3075_3103_F	GATGACTTTTTAGCTAATGGTCAGGCAGC	108	SP101_SPET11_3168_3196_R	AATCGACGACCATCTTGGAAAGATTTCTC	715
97	SP101_SPET11_3386_3403_F	AGCGTAAAGGTGAACCTT	25	SP101_SPET11_3480_3506_R	CCAGCAGTTACTGTCCCCTCATCTTTG	769
98	SP101_SPET11_3511_3535_F	GCTTCAGGAATCAATGATGGAGCAG	116	SP101_SPET11_3605_3629_R	GGGTCTACACCTGCACTTGCATAAC	832
111	RPOB_EC_3775_3803_F	CTTGGAGGTAAGTCTCATTTTGGTGGGCA	87	RPOB_EC_3829_3858_R	CGTATAAGCTGCACCATAAGCTTGTAATGC	797
112	VALS_EC_1833_1850_F	CGACGCGCTGCGCTTCAC	65	VALS_EC_1920_1943_R	GCGTTCCACAGCTTGTTGCAGAAG	822
113	RPOB_EC_1336_1353_F	GACCACCTCGGCAACCGT	97	RPOB_EC_1438_1455_R	TTCGCTCTCGGCCTGGCC	1386
114	TUFB_EC_225_251_F	GCACTATGCACACGTAGATTGTCCTGG	111	TUFB_EC_284_309_R	TATAGCACCATCCATCTGAGCGGCAC	930
115	DNAK_EC_428_449_F	CGGCGTACTTCAACGACAGCCA	72	DNAK_EC_503_522_R	CGCGGTCGGCTCGTTGATGA	792
116	VALS_EC_1920_1943_F	CTTCTGCAACAAGCTGTGGAACGC	85	VALS_EC_1948_1970_R	TCGCAGTTCATCAGCACGAAGCG	1075
117	TUFB_EC_757_774_F	AAGACGACCTGCACGGGC	6	TUFB_EC_849_867_R	GCGCTCCACGTCTTCACGC	819
118	23S_EC_2646_2667_F	CTGTTCTTAGTACGAGAGGACC	84	23S_EC_2745_2765_R	TTCGTGCTTAGATGCTTTCAG	1389
119	16S_EC_969_985_1P_F	ACGCGAAGAACCTTACpC	19	16S_EC_1061_1078_2P_R	ACGACACGAGCpTpGACGAC	733
120	16S_EC_972_985_2P_F	CGAAGAACpCpTTACC	63	16S_EC_1064_1075_2P_R	ACACGAGCpTpGAC	727
121	16S_EC_972_985_F	CGAAGAACCTTACC	63	16S_EC_1064_1075_R	ACACGAGCTGAC	727
122	TRNA_ILE-RRNH_EC_32_50.2_F	CCTGATAAGGGTGAGGTCG	61	23S_EC_40_59_R	ACGTCCTTCATCGCCTCTGA	740

123	23S_EC_-7_15_F	GTTGTGAGGTTAAGCGACTAAG	140	23S_EC_430_450_R	CTATCGGTCAGTCAGGAGTAT	799
124	23S_EC_-7_15_F	GTTGTGAGGTTAAGCGACTAAG	141	23S_EC_891_910_R	TTGCATCGGGTTGGTAAGTC	1403
125	23S_EC_430_450_F	ATACTCCTGACTGACCGATAG	30	23S_EC_1424_1442_R	AACATAGCCTTCTCCGTCC	712
126	23S_EC_891_910_F	GACTTACCAACCCGATGCAA	100	23S_EC_1908_1931_R	TACCTTAGGACCGTTATAGTTACG	893
127	23S_EC_1424_1442_F	GGACGGAGAAGGCTATGTT	117	23S_EC_2475_2494_R	CCAAACACCGCCGTCGATAT	765
128	23S_EC_1908_1931_F	CGTAACTATAACGGTCCTAAGGTA	73	23S_EC_2833_2852_R	GCTTACACACCCGGCCTATC	826
129	23S_EC_2475_2494_F	ATATCGACGGCGGTGTTTGG	31	TRNA_ASP-RRNH_EC_23_41.2_R	GCGTGACAGGCAGGTATTC	820
131	16S_EC_-60_-39_F	AGTCTCAAGAGTGAACACGTAA	28	16S_EC_508_525_R	GCTGCTGGCACGGAGTTA	823
132	16S_EC_326_345_F	GACACGGTCCAGACTCCTAC	95	16S_EC_1041_1058_R	CCATGCAGCACCTGTCTC	771
133	16S_EC_705_724_F	GATCTGGAGGAATACCGGTG	107	16S_EC_1493_1512_R	ACGGTTACCTTGTTACGACT	739
134	16S_EC_1268_1287_F	GAGAGCAAGCGGACCTCATA	101	TRNA_ALA-RRNH_EC_30_46.2_R	CCTCCTGCGTGCAAAGC	780
135	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_R	ACAACACGAGCTGACGAC	719
137	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_I14_R	ACAACACGAGCTGICGAC	721
138	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_I12_R	ACAACACGAGCIGACGAC	718
139	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_I11_R	ACAACACGAGITGACGAC	722
140	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_I16_R	ACAACACGAGCTGACIAC	720
141	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_2I_R	ACAACACGAICTIACGAC	723
142	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_3I_R	ACAACACIAICTIACGAC	724
143	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1061_1078.2_4I_R	ACAACACIAICTIACIAC	725
147	23S_EC_2652_2669_F	CTAGTACGAGAGGACCGG	79	23S_EC_2741_2760_R	ACTTAGATGCTTTCAGCGGT	743
158	16S_EC_683_700_F	GTGTAGCGGTGAAATGCG	137	16S_EC_880_894_R	CGTACTCCCCAGGCG	796
159	16S_EC_1100_1116_F	CAACGAGCGCAACCCTT	42	16S_EC_1174_1188_R	TCCCCACCTTCCTCC	1019
215	SSPE_BA_121_137_F	AACGCACAATCAGAAGC	3	SSPE_BA_197_216_R	TCTGTTTCAGTTGCAAATTC	1132
220	GROL_EC_941_959_F	TGGAAGATCTGGGTCAGGC	544	GROL_EC_1039_1060_R	CAATCTGCTGACGGATCTGAGC	759
221	INFB_EC_1103_1124_F	GTCGTGAAAACGAGCTGGAAGA	133	INFB_EC_1174_1191_R	CATGATGGTCACAACCGG	764
222	HFLB_EC_1082_1102_F	TGGCGAACCTGGTGAACGAAGC	569	HFLB_EC_1144_1168_R	CTTTCGCTTTCTCGAACTCAACCAT	802
223	INFB_EC_1969_1994_F	CGTCAGGGTAAATTCCGTGAAGTTAA	74	INFB_EC_2038_2058_R	AACTTCGCCTTCGGTCATGTT	713
224	GROL_EC_219_242_F	GGTGAAAGAAGTTGCCTCTAAAGC	128	GROL_EC_328_350_R	TTCAGGTCCATCGGGTTCATGCC	1377
225	VALS_EC_1105_1124_F	CGTGGCGGCGTGGTTATCGA	77	VALS_EC_1195_1214_R	ACGAACTGGATGTCGCCGTT	732
226	16S_EC_556_575_F	CGGAATTACTGGGCGTAAAG	70	16S_EC_683_700_R	CGCATTTCACCGCTACAC	791
227	RPOC_EC_1256_1277_F	ACCCAGTGCTGCTGAACCGTGC	16	RPOC_EC_1295_1315_R	GTTCAAATGCCTGGATACCCA	843
228	16S_EC_774_795_F	GGGAGCAAACAGGATTAGATAC	122	16S_EC_880_894_R	CGTACTCCCCAGGCG	796
229	RPOC_EC_1584_1604_F	TGGCCCGAAAGAAGCTGAGCG	567	RPOC_EC_1623_1643_R	ACGCGGGCATGCAGAGATGCC	737
230	16S_EC_1082_1100_F	ATGTTGGGTTAAGTCCCGC	37	16S_EC_1177_1196_R	TGACGTCATCCCCACCTTCC	1158
231	16S_EC_1389_1407_F	CTTGTACACACCGCCCGTC	88	16S_EC_1525_1541_R	AAGGAGGTGATCCAGCC	714

232	16S_EC_1303_1323_F	CGGATTGGAGTCTGCAACTCG	71	16S_EC_1389_1407_R	GACGGGCGGTGTGTACAAG	808
233	23S_EC_23_37_F	GGTGGATGCCTTGGC	129	23S_EC_115_130_R	GGGTTTCCCCATTCGG	833
234	23S_EC_187_207_F	GGGAACTGAAACATCTAAGTA	121	23S_EC_242_256_R	TTCGCTCGCCGCTAC	1385
235	23S_EC_1602_1620_F	TACCCCAAACCGACACAGG	184	23S_EC_1686_1703_R	CCTTCTCCCGAAGTTACG	782
236	23S_EC_1685_1703_F	CCGTAACTTCGGGAGAAGG	58	23S_EC_1828_1842_R	CACCGGGCAGGCGTC	760
237	23S_EC_1827_1843_F	GACGCCTGCCCGGTGC	99	23S_EC_1929_1949_R	CCGACAAGGAATTTCGCTACC	775
238	23S_EC_2434_2456_F	AAGGTACTCCGGGGATAACAGGC	9	23S_EC_2490_2511_R	AGCCGACATCGAGGTGCCAAAC	746
239	23S_EC_2599_2616_F	GACAGTTCGGTCCCTATC	96	23S_EC_2653_2669_R	CCGGTCCTCTCGTACTA	777
240	23S_EC_2653_2669_F	TAGTACGAGAGGACCGG	227	23S_EC_2737_2758_R	TTAGATGCTTTCAGCACTTATC	1369
241	23S_BS_-68_-44_F	AAACTAGATAACAGTAGACATCAC	1	23S_BS_5_21_R	GTGCGCCCTTTCTAACTT	841
242	16S_EC_8_27_F	AGAGTTTGATCATGGCTCAG	23	16S_EC_342_358_R	ACTGCTGCCTCCCGTAG	742
243	16S_EC_314_332_F	CACTGGAACTGAGACACGG	48	16S_EC_556_575_R	CTTTACGCCCAGTAATTCCG	801
244	16S_EC_518_536_F	CCAGCAGCCGCGGTAATAC	57	16S_EC_774_795_R	GTATCTAATCCTGTTTGCTCCC	839
245	16S_EC_683_700_F	GTGTAGCGGTGAAATGCG	137	16S_EC_967_985_R	GGTAAGGTTCTTCGCGTTG	835
246	16S_EC_937_954_F	AAGCGGTGGAGCATGTGG	7	16S_EC_1220_1240_R	ATTGTAGCACGTGTGTAGCCC	757
247	16S_EC_1195_1213_F	CAAGTCATCATGGCCCTTA	46	16S_EC_1525_1541_R	AAGGAGGTGATCCAGCC	714
248	16S_EC_8_27_F	AGAGTTTGATCATGGCTCAG	23	16S_EC_1525_1541_R	AAGGAGGTGATCCAGCC	714
249	23S_EC_1831_1849_F	ACCTGCCCAGTGCTGGAAG	18	23S_EC_1919_1936_R	TCGCTACCTTAGGACCGT	1080
250	16S_EC_1387_1407_F	GCCTTGTACACACCTCCCGTC	112	16S_EC_1494_1513_R	CACGGCTACCTTGTTACGAC	761
251	16S_EC_1390_1411_F	TTGTACACACCGCCCGTCATAC	693	16S_EC_1486_1505_R	CCTTGTTACGACTTCACCCC	783
252	16S_EC_1367_1387_F	TACGGTGAATACGTTCCCGGG	191	16S_EC_1485_1506_R	ACCTTGTTACGACTTCACCCCA	731
253	16S_EC_804_822_F	ACCACGCCGTAAACGATGA	14	16S_EC_909_929_R	CCCCCGTCAATTCCTTTGAGT	773
254	16S_EC_791_812_F	GATACCCTGGTAGTCCACACCG	106	16S_EC_886_904_R	GCCTTGCGACCGTACTCCC	817
255	16S_EC_789_810_F	TAGATACCCTGGTAGTCCACGC	206	16S_EC_882_899_R	GCGACCGTACTCCCCAGG	818
256	16S_EC_1092_1109_F	TAGTCCCGCAACGAGCGC	228	16S_EC_1174_1195_R	GACGTCATCCCCACCTTCCTCC	810
257	23S_EC_2586_2607_F	TAGAACGTCGCGAGACAGTTCG	203	23S_EC_2658_2677_R	AGTCCATCCCGGTCCTCTCG	749
258	RNASEP_SA_31_49_F	GAGGAAAGTCCATGCTCAC	103	RNASEP_SA_358_379_R	ATAAGCCATGTTCTGTTCCATC	750
258	RNASEP_SA_31_49_F	GAGGAAAGTCCATGCTCAC	103	RNASEP_EC_345_362_R	ATAAGCCGGGTTCTGTCG	751
258	RNASEP_SA_31_49_F	GAGGAAAGTCCATGCTCAC	103	RNASEP_BS_363_384_R	GTAAGCCATGTTTTGTTCCATC	838
258	RNASEP_BS_43_61_F	GAGGAAAGTCCATGCTCGC	104	RNASEP_SA_358_379_R	ATAAGCCATGTTCTGTTCCATC	750
258	RNASEP_BS_43_61_F	GAGGAAAGTCCATGCTCGC	104	RNASEP_EC_345_362_R	ATAAGCCGGGTTCTGTCG	751
258	RNASEP_BS_43_61_F	GAGGAAAGTCCATGCTCGC	104	RNASEP_BS_363_384_R	GTAAGCCATGTTTTGTTCCATC	838
258	RNASEP_EC_61_77_F	GAGGAAAGTCCGGGCTC	105	RNASEP_SA_358_379_R	ATAAGCCATGTTCTGTTCCATC	750
258	RNASEP_EC_61_77_F	GAGGAAAGTCCGGGCTC	105	RNASEP_EC_345_362_R	ATAAGCCGGGTTCTGTCG	751
258	RNASEP_EC_61_77_F	GAGGAAAGTCCGGGCTC	105	RNASEP_BS_363_384_R	GTAAGCCATGTTTTGTTCCATC	838
259	RNASEP_BS_43_61_F	GAGGAAAGTCCATGCTCGC	104	RNASEP_BS_363_384_R	GTAAGCCATGTTTTGTTCCATC	838

260	RNASEP_EC_61_77_F	GAGGAAAGTCCGGGCTC	105	RNASEP_EC_345_362_R	ATAAGCCGGGTTCTGTCG	751
262	RNASEP_SA_31_49_F	GAGGAAAGTCCATGCTCAC	103	RNASEP_SA_358_379_R	ATAAGCCATGTTCTGTTCCATC	750
263	16S_EC_1082_1100_F	ATGTTGGGTTAAGTCCCGC	37	16S_EC_1525_1541_R	AAGGAGGTGATCCAGCC	714
264	16S_EC_556_575_F	CGGAATTACTGGGCGTAAAG	70	16S_EC_774_795_R	GTATCTAATCCTGTTTGCTCCC	839
265	16S_EC_1082_1100_F	ATGTTGGGTTAAGTCCCGC	37	16S_EC_1177_1196_10G_R	TGACGTCATGCCCACCTTCC	1160
266	16S_EC_1082_1100_F	ATGTTGGGTTAAGTCCCGC	37	16S_EC_1177_1196_10G_11G_R	TGACGTCATGGCCACCTTCC	1161
268	YAED_EC_513_532_F_MOD	GGTGTTAAATAGCCTGGCAG	130	TRNA_ALA-RRNH_EC_30_49_F_MOD	AGACCTCCTGCGTGCAAAGC	744
269	16S_EC_1082_1100_F_MOD	ATGTTGGGTTAAGTCCCGC	37	16S_EC_1177_1196_R_MOD	TGACGTCATCCCCACCTTCC	1158
270	23S_EC_2586_2607_F_MOD	TAGAACGTCGCGAGACAGTTCG	203	23S_EC_2658_2677_R_MOD	AGTCCATCCCGGTCCTCTCG	749
272	16S_EC_969_985_F	ACGCGAAGAACCTTACC	19	16S_EC_1389_1407_R	GACGGGCGGTGTGTACAAG	807
273	16S_EC_683_700_F	GTGTAGCGGTGAAATGCG	137	16S_EC_1303_1323_R	CGAGTTGCAGACTGCGATCCG	788
274	16S_EC_49_68_F	TAACACATGCAAGTCGAACG	152	16S_EC_880_894_R	CGTACTCCCCAGGCG	796
275	16S_EC_49_68_F	TAACACATGCAAGTCGAACG	152	16S_EC_1061_1078_R	ACGACACGAGCTGACGAC	734
277	CYA_BA_1349_1370_F	ACAACGAAGTACAATACAAGAC	12	CYA_BA_1426_1447_R	CTTCTACATTTTTAGCCATCAC	800
278	16S_EC_1090_1111_2_F	TTAAGTCCCGCAACGAGCGCAA	650	16S_EC_1175_1196_R	TGACGTCATCCCCACCTTCCTC	1159
279	16S_EC_405_432_F	TGAGTGATGAAGGCCTTAGGGTTGTAAA	464	16S_EC_507_527_R	CGGCTGCTGGCACGAAGTTAG	793
280	GROL_EC_496_518_F	ATGGACAAGGTTGGCAAGGAAGG	34	GROL_EC_577_596_R	TAGCCGCGGTCGAATTGCAT	914
281	GROL_EC_511_536_F	AAGGAAGGCGTGATCACCGTTGAAGA	8	GROL_EC_571_593_R	CCGCGGTCGAATTGCATGCCTTC	776
288	RPOB_EC_3802_3821_F	CAGCGTTTCGGCGAAATGGA	51	RPOB_EC_3862_3885_R	CGACTTGACGGTTAACATTTCCTG	786
289	RPOB_EC_3799_3821_F	GGGCAGCGTTTCGGCGAAATGGA	124	RPOB_EC_3862_3888_R	GTCCGACTTGACGGTCAACATTTCCTG	840
290	RPOC_EC_2146_2174_F	CAGGAGTCGTTCAACTCGATCTACATGAT	52	RPOC_EC_2227_2245_R	ACGCCATCAGGCCACGCAT	736
291	ASPS_EC_405_422_F	GCACAACCTGCGGCTGCG	110	ASPS_EC_521_538_R	ACGGCACGAGGTAGTCGC	738
292	RPOC_EC_1374_1393_F	CGCCGACTTCGACGGTGACC	69	RPOC_EC_1437_1455_R	GAGCATCAGCGTGCGTGCT	811
293	TUFB_EC_957_979_F	CCACACGCCGTTCTTCAACAACT	55	TUFB_EC_1034_1058_R	GGCATCACCATTTCCTTGTCCTTCG	829
294	16S_EC_7_33_F	GAGAGTTTGATCCTGGCTCAGAACGAA	102	16S_EC_101_122_R	TGTTACTCACCCGTCTGCCACT	1345
295	VALS_EC_610_649_F	ACCGAGCAAGGAGACCAGC	17	VALS_EC_705_727_R	TATAACGCACATCGTCAGGGTGA	929
344	16S_EC_971_990_F	GCGAAGAACCTTACCAGGTC	113	16S_EC_1043_1062_R	ACAACCATGCACCACCTGTC	726
346	16S_EC_713_732_TMOD_F	TAGAACACCGATGGCGAAGGC	202	16S_EC_789_809_TMOD_R	TCGTGGACTACCAGGGTATCTA	1110
347	16S_EC_785_806_TMOD_F	TGGATTAGAGACCCTGGTAGTCC	560	16S_EC_880_897_TMOD_R	TGGCCGTACTCCCCAGGCG	1278
348	16S_EC_960_981_TMOD	TTTCGATGCAACGCGAAGAACCT	706	16S_EC_1054_1073_TMOD_R	TACGAGCTGACGACAGCCATG	895

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349	23S_EC_1826_1843_TMOD_F	TCTGACACCTGCCCGGTGC	401	23S_EC_1906_1924_TMOD_R	TGACCGTTATAGTTACGGCC	1156
350	CAPC_BA_274_303_TMOD_F	TGATTATTGTTATCCTGTTATGCCATTTGAG	476	CAPC_BA_349_376_TMOD_R	TGTAACCCTTGTCTTTGAATTGTATTTGC	1314
351	CYA_BA_1353_1379_TMOD_F	TCGAAGTACAATACAAGACAAAAGAAGG	355	CYA_BA_1448_1467_TMOD_R	TTGTTAACGGCTTCAAGACCC	1423
352	INFB_EC_1365_1393_TMOD_F	TTGCTCGTGGTGCACAAGTAACGGATATTA	687	INFB_EC_1439_1467_TMOD_R	TTGCTGCTTTCGCATGGTTAATTGCTTCAA	1411
353	LEF_BA_756_781_TMOD_F	TAGCTTTTGCATATTATATCGAGCCAC	220	LEF_BA_843_872_TMOD_R	TTCTTCCAAGGATAGATTTATTTCTTGTTCG	1394
354	RPOC_EC_2218_2241_TMOD_F	TCTGGCAGGTATGCGTGGTCTGATG	405	RPOC_EC_2313_2337_TMOD_R	TCGCACCGTGGGTTGAGATGAAGTAC	1072
355	SSPE_BA_115_137_TMOD_F	TCAAGCAAACGCACAATCAGAAGC	255	SSPE_BA_197_222_TMOD_R	TTGCACGTCTGTTTCAGTTGCAAATTC	1402
356	RPLB_EC_650_679_TMOD_F	TGACCTACAGTAAGAGGTTCTGTAATGAACC	449	RPLB_EC_739_762_TMOD_R	TTCCAAGTGCTGGTTTACCCCATGG	1380
357	RPLB_EC_688_710_TMOD_F	TCATCCACACGGTGGTGGTGAAGG	296	RPLB_EC_736_757_TMOD_R	TGTGCTGGTTTACCCCATGGAGT	1337
358	VALS_EC_1105_1124_TMOD_F	TCGTGGCGGCGTGGTTATCGA	385	VALS_EC_1195_1218_TMOD_R	TCGGTACGAACTGGATGTCGCCGTT	1093
359	RPOB_EC_1845_1866_TMOD_F	TTATCGCTCAGGCGAACTCCAAC	659	RPOB_EC_1909_1929_TMOD_R	TGCTGGATTCGCCTTTGCTACG	1250
360	23S_EC_2646_2667_TMOD_F	TCTGTTCTTAGTACGAGAGGACC	409	23S_EC_2745_2765_TMOD_R	TTTCGTGCTTAGATGCTTTCAG	1434
361	16S_EC_1090_1111_2_TMOD_F	TTTAAGTCCCGCAACGAGCGCAA	697	16S_EC_1175_1196_TMOD_R	TTGACGTCATCCCCACCTTCCTC	1398
362	RPOB_EC_3799_3821_TMOD_F	TGGGCAGCGTTTCGGCGAAATGGA	581	RPOB_EC_3862_3888_TMOD_R	TGTCCGACTTGACGGTCAACATTTCCTG	1325
363	RPOC_EC_2146_2174_TMOD_F	TCAGGAGTCGTTCAACTCGATCTACATGAT	284	RPOC_EC_2227_2245_TMOD_R	TACGCCATCAGGCCACGCAT	898
364	RPOC_EC_1374_1393_TMOD_F	TCGCCGACTTCGACGGTGACC	367	RPOC_EC_1437_1455_TMOD_R	TGAGCATCAGCGTGCGTGCT	1166
367	TUFB_EC_957_979_TMOD_F	TCCACACGCCGTTCTTCAACAACT	308	TUFB_EC_1034_1058_TMOD_R	TGGCATCACCATTTCCTTGTCCTTCG	1276
423	SP101_SPET11_893_921_TMOD_F	TGGGCAACAGCAGCGGATTGCGATTGCGCG	580	SP101_SPET11_988_1012_TMOD_R	TCATGACAGCCAAGACCTCACCCACC	990
424	SP101_SPET11_1154_1179_TMOD_F	TCAATACCGCAACAGCGGTGGCTTGGG	258	SP101_SPET11_1251_1277_TMOD_R	TGACCCCAACCTGGCCTTTTGTCGTTGA	1155
425	SP101_SPET11_118_147_TMOD_F	TGCTGGTGAAAATAACCCAGATGTCGTCTTC	528	SP101_SPET11_213_238_TMOD_R	TTGTGGCCGATTTCACCACCTGCTCCT	1422
426	SP101_SPET11_1314_1336_TMOD_F	TCGCAAAAAAATCCAGCTATTAGC	363	SP101_SPET11_1403_1431_TMOD_R	TAAACTATTTTTTTAGCTATACTCGAACAC	849
427	SP101_SPET11_1408_1437_TMOD_F	TCGAGTATAGCTAAAAAAATAGTTTATGACA	359	SP101_SPET11_1486_1515_TMOD_R	TGGATAATTGGTCGTAACAAGGGATAGTGAG	1268

428	SP101_SPET11_1688_1716_TMOD_F	TCCTATATTAATCGTTTACAGAAACTGGCT	334	SP101_SPET11_1783_1808_TMOD_R	TATATGATTATCATTGAACTGCGGCCG	932
429	SP101_SPET11_1711_1733_TMOD_F	TCTGGCTAAAACTTTGGCAACGGT	406	SP101_SPET11_1808_1835_TMOD_R	TGCGTGACGACCTTCTTGAATTGTAATCA	1239
430	SP101_SPET11_1807_1835_TMOD_F	TATGATTACAATTCAAGAAGGTCGTCACGC	235	SP101_SPET11_1901_1927_TMOD_R	TTTGGACCTGTAATCAGCTGAATACTGG	1439
431	SP101_SPET11_1967_1991_TMOD_F	TTAACGGTTATCATGGCCCAGATGGG	649	SP101_SPET11_2062_2083_TMOD_R	TATTGCCCAGAAATCAAATCATC	940
432	SP101_SPET11_216_243_TMOD_F	TAGCAGGTGGTGAAATCGGCCACATGATT	210	SP101_SPET11_308_333_TMOD_R	TTGCCACTTTGACAACTCCTGTTGCTG	1404
433	SP101_SPET11_2260_2283_TMOD_F	TCAGAGACCGTTTTATCCTATCAGC	272	SP101_SPET11_2375_2397_TMOD_R	TTCTGGGTGACCTGGTGTTTTAGA	1393
434	SP101_SPET11_2375_2399_TMOD_F	TTCTAAAACACCAGGTCACCCAGAAG	675	SP101_SPET11_2470_2497_TMOD_R	TAGCTGCTAGATGAGCTTCTGCCATGGCC	918
435	SP101_SPET11_2468_2487_TMOD_F	TATGGCCATGGCAGAAGCTCA	238	SP101_SPET11_2543_2570_TMOD_R	TCCATAAGGTCACCGTCACCATTCAAAGC	1007
436	SP101_SPET11_266_295_TMOD_F	TCTTGTACTTGTGGCTCACACGGCTGTTTGG	417	SP101_SPET11_355_380_TMOD_R	TGCTGCTTTGATGGCTGAATCCCCTTC	1249
437	SP101_SPET11_2961_2984_TMOD_F	TACCATGACAGAAGGCATTTTGACA	183	SP101_SPET11_3023_3045_TMOD_R	TGGAATTTACCAGCGATAGACACC	1264
438	SP101_SPET11_3075_3103_TMOD_F	TGATGACTTTTTAGCTAATGGTCAGGCAGC	473	SP101_SPET11_3168_3196_TMOD_R	TAATCGACGACCATCTTGGAAAGATTTCTC	875
439	SP101_SPET11_322_344_TMOD_F	TGTCAAAGTGGCACGTTTACTGGC	631	SP101_SPET11_423_441_TMOD_R	TATCCCCTGCTTCTGCTGCC	934
440	SP101_SPET11_3386_3403_TMOD_F	TAGCGTAAAGGTGAACCTT	215	SP101_SPET11_3480_3506_TMOD_R	TCCAGCAGTTACTGTCCCCTCATCTTTG	1005
441	SP101_SPET11_3511_3535_TMOD_F	TGCTTCAGGAATCAATGATGGAGCAG	531	SP101_SPET11_3605_3629_TMOD_R	TGGGTCTACACCTGCACTTGCATAAC	1294
442	SP101_SPET11_358_387_TMOD_F	TGGGGATTCAGCCATCAAAGCAGCTATTGAC	588	SP101_SPET11_448_473_TMOD_R	TCCAACCTTTTCCACAACAGAATCAGC	998
443	SP101_SPET11_600_629_TMOD_F	TCCTTACTTCGAACTATGAATCTTTTGGAAG	348	SP101_SPET11_686_714_TMOD_R	TCCCATTTTTTCACGCATGCTGAAAATATC	1018
444	SP101_SPET11_658_684_TMOD_F	TGGGGATTGATATCACCGATAAGAAGAA	589	SP101_SPET11_756_784_TMOD_R	TGATTGGCGATAAAGTGATATTTTCTAAAA	1189
445	SP101_SPET11_776_801_TMOD_F	TTCGCCAATCAAAACTAAGGGAATGGC	673	SP101_SPET11_871_896_TMOD_R	TGCCCACCAGAAAGACTAGCAGGATAA	1217
446	SP101_SPET11_1_29_TMOD_F	TAACCTTAATTGGAAAGAAACCCAAGAAGT	154	SP101_SPET11_92_116_TMOD_R	TCCTACCCAACGTTCACCAAGGGCAG	1044
447	SP101_SPET11_364_385_F	TCAGCCATCAAAGCAGCTATTG	276	SP101_SPET11_448_471_R	TACCTTTTCCACAACAGAATCAGC	894
448	SP101_SPET11_3085_3104_F	TAGCTAATGGTCAGGCAGCC	216	SP101_SPET11_3170_3194_R	TCGACGACCATCTTGGAAAGATTTC	1066
449	RPLB_EC_690_710_F	TCCACACGGTGGTGGTGAAGG	309	RPLB_EC_737_758_R	TGTGCTGGTTTACCCCATGGAG	1336
481	BONTA_X52066_538_552_F	TATGGCTCTACTCAA	239	BONTA_X52066_647_660_R	TGTTACTGCTGGAT	1346

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482	BONTA_X52066_538_552P_F	TA*TpGGC*Tp*Cp*TpA*Cp*Tp*CpAA	143	BONTA_X52066_647_660P_R	TG*Tp*TpA*Cp*TpG*Cp*TpGGAT	1146
483	BONTA_X52066_701_720_F	GAATAGCAATTAATCCAAAT	94	BONTA_X52066_759_775_R	TTACTTCTAACCCACTC	1367
484	BONTA_X52066_701_720P_F	GAA*TpAG*CpAA*Tp*TpAA*Tp*Cp*CpAAAT	91	BONTA_X52066_759_775P_R	TTA*Cp*Tp*Tp*Cp*TpAA*Cp*Cp*CpA*Cp*TpC	1359
485	BONTA_X52066_450_473_F	TCTAGTAATAATAGGACCCTCAGC	393	BONTA_X52066_517_539_R	TAACCATTTCGCGTAAGATTCAA	859
486	BONTA_X52066_450_473P_F	T*Cp*TpAGTAATAATAGGA*Cp*Cp*Cp*Tp*CpAGC	142	BONTA_X52066_517_539P_R	TAACCA*Tp*Tp*Tp*CpGCGTAAGA*Tp*Tp*CpAA	857
487	BONTA_X52066_591_620_F	TGAGTCACTTGAAGTTGATACAAATCCTCT	463	BONTA_X52066_644_671_R	TCATGTGCTAATGTTACTGCTGGATCTG	992
608	SSPE_BA_156_168P_F	TGGTpGCpTpAGCpATT	616	SSPE_BA_243_255P_R	TGCpAGCpTGATpTpGT	1241
609	SSPE_BA_75_89P_F	TACpAGAGTpTpTpGCpGAC	192	SSPE_BA_163_177P_R	TGTGCTpTpTpGAATpGCpT	1338
610	SSPE_BA_150_168P_F	TGCTTCTGGTpGCpTpAGCpATT	533	SSPE_BA_243_264P_R	TGATTGTTTTGCpAGCpTGATpTpGT	1191
611	SSPE_BA_72_89P_F	TGGTACpAGAGTpTpTpGCpGAC	602	SSPE_BA_163_182P_R	TCATTTGTGCTpTpTpGAATpGCpT	995
612	SSPE_BA_114_137P_F	TCAAGCAAACGCACAATpCpAGAAGC	255	SSPE_BA_196_222P_R	TTGCACGTCpTpGTTTCAGTTGCAAATTC	1401
699	SSPE_BA_123_153_F	TGCACAATCAGAAGCTAAGAAAGCGCAAGCT	488	SSPE_BA_202_231_R	TTTCACAGCATGCACGTCTGTTTCAGTTGC	1431
700	SSPE_BA_156_168_F	TGGTGCTAGCATT	612	SSPE_BA_243_255_R	TGCAGCTGATTGT	1202
701	SSPE_BA_75_89_F	TACAGAGTTTGCGAC	179	SSPE_BA_163_177_R	TGTGCTTTGAATGCT	1338
702	SSPE_BA_150_168_F	TGCTTCTGGTGCTAGCATT	533	SSPE_BA_243_264_R	TGATTGTTTTGCAGCTGATTGT	1190
703	SSPE_BA_72_89_F	TGGTACAGAGTTTGCGAC	600	SSPE_BA_163_182_R	TCATTTGTGCTTTGAATGCT	995
704	SSPE_BA_146_168_F	TGCAAGCTTCTGGTGCTAGCATT	484	SSPE_BA_242_267_R	TTGTGATTGTTTTGCAGCTGATTGTG	1421
705	SSPE_BA_63_89_F	TGCTAGTTATGGTACAGAGTTTGCGAC	518	SSPE_BA_163_191_R	TCATAACTAGCATTTGTGCTTTGAATGCT	986
706	SSPE_BA_114_137_F	TCAAGCAAACGCACAATCAGAAGC	255	SSPE_BA_196_222_R	TTGCACGTCTGTTTCAGTTGCAAATTC	1402
770	PLA_AF053945_7377_7402_F	TGACATCCGGCTCACGTTATTATGGT	442	PLA_AF053945_7434_7462_R	TGTAAATTCCGCAAAGACTTTGGCATTAG	1313
771	PLA_AF053945_7382_7404_F	TCCGGCTCACGTTATTATGGTAC	327	PLA_AF053945_7482_7502_R	TGGTCTGAGTACCTCCTTTGC	1304
772	PLA_AF053945_7481_7503_F	TGCAAAGGAGGTACTCAGACCAT	481	PLA_AF053945_7539_7562_R	TATTGGAAATACCGGCAGCATCTC	943
773	PLA_AF053945_7186_7211_F	TTATACCGGAAACTTCCCGAAAGGAG	657	PLA_AF053945_7257_7280_R	TAATGCGATACTGGCCTGCAAGTC	879
774	CAF1_AF053947_33407_33430_F	TCAGTTCCGTTATCGCCATTGCAT	292	CAF1_AF053947_33494_33514_R	TGCGGGCTGGTTCAACAAGAG	1235
775	CAF1_AF053947_33515_33541_F	TCACTCTTACATATAAGGAAGGCGCTC	270	CAF1_AF053947_33595_33621_R	TCCTGTTTTATAGCCGCCAAGAGTAAG	1053
776	CAF1_AF053947_33435_33457_F	TGGAACTATTGCAACTGCTAATG	542	CAF1_AF053947_33499_33517_R	TGATGCGGGCTGGTTCAAC	1183

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777	CAF1_AF053947_33687_33716_F	TCAGGATGGAAATAACCACCAATTCACTAC	286	CAF1_AF053947_33755_33782_R	TCAAGGTTCTCACCGTTTACCTTAGGAG	962
778	INV_U22457_515_539_F	TGGCTCCTTGGTATGACTCTGCTTC	573	INV_U22457_571_598_R	TGTTAAGTGTGTTGCGGCTGTCTTTATT	1343
779	INV_U22457_699_724_F	TGCTGAGGCCTGGACCGATTATTTAC	525	INV_U22457_753_776_R	TCACGCGACGAGTGCCATCCATTG	976
780	INV_U22457_834_858_F	TTATTTACCTGCACTCCCACAACTG	664	INV_U22457_942_966_R	TGACCCAAAGCTGAAAGCTTTACTG	1154
781	INV_U22457_1558_1581_F	TGGTAACAGAGCCTTATAGGCGCA	597	INV_U22457_1619_1643_R	TTGCGTTGCAGATTATCTTTACCAA	1408
782	LL_NC003143_2366996_2367019_F	TGTAGCCGCTAAGCACTACCATCC	627	LL_NC003143_2367073_2367097_R	TCTCATCCCGATATTACCGCCATGA	1123
783	LL_NC003143_2367172_2367194_F	TGGACGGCATCACGATTCTCTAC	550	LL_NC003143_2367249_2367271_R	TGGCAACAGCTCAACACCTTTGG	1272
874	RPLB_EC_649_679_F	TGICCIACIGTIIGIGGTTCTGTAATGAACC	620	RPLB_EC_739_762_TMOD_R	TTCCAAGTGCTGGTTTACCCCATGG	1380
875	RPLB_EC_642_679P_F	TpCpCpTpTpGITpGICCIACIGTIIGIGGTTCTGTAATGAACC	646	RPLB_EC_739_762_TMOD_R	TTCCAAGTGCTGGTTTACCCCATGG	1380
876	MECIA_Y14051_3315_3341_F	TTACACATATCGTGAGCAATGAACTGA	653	MECIA_Y14051_3367_3393_R	TGTGATATGGAGGTGTAGAAGGTGTTA	1333
877	MECA_Y14051_3774_3802_F	TAAAACAAACTACGGTAACATTGATCGCA	144	MECA_Y14051_3828_3854_R	TCCCAATCTAACTTCCACATACCATCT	1015
878	MECA_Y14051_3645_3670_F	TGAAGTAGAAATGACTGAACGTCCGA	434	MECA_Y14051_3690_3719_R	TGATCCTGAATGTTTATATCTTTAACGCCT	1181
879	MECA_Y14051_4507_4530_F	TCAGGTACTGCTATCCACCCTCAA	288	MECA_Y14051_4555_4581_R	TGGATAGACGTCATATGAAGGTGTGCT	1269
880	MECA_Y14051_4510_4530_F	TGTACTGCTATCCACCCTCAA	626	MECA_Y14051_4586_4610_R	TATTCTTCGTTACTCATGCCATACA	939
881	MECA_Y14051_4669_4698_F	TCACCAGGTTCAACTCAAAAAATATTAACA	262	MECA_Y14051_4765_4793_R	TAACCACCCCAAGATTTATCTTTTTGCCA	858
882	MECA_Y14051_4520_4530P_F	TCpCpACpCpCpTpCpAA	389	MECA_Y14051_4590_4600P_R	TpACpTpCpATpGCpCpA	1357
883	MECA_Y14051_4520_4530P_F	TCpCpACpCpCpTpCpAA	389	MECA_Y14051_4600_4610P_R	TpATpTpCpTpTpCpGTpT	1358
902	TRPE_AY094355_1467_1491_F	ATGTCGATTGCAATCCGTACTTGTG	36	TRPE_AY094355_1569_1592_R	TGCGCGAGCTTTTATTTGGGTTTC	1231
903	TRPE_AY094355_1445_1471_F	TGGATGGCATGGTGAAATGGATATGTC	557	TRPE_AY094355_1551_1580_R	TATTTGGGTTTCATTCCACTCAGATTCTGG	944
904	TRPE_AY094355_1278_1303_F	TCAAATGTACAAGGTGAAGTGCGTGA	247	TRPE_AY094355_1392_1418_R	TCCTCTTTTCACAGGCTCTACTTCATC	1048
905	TRPE_AY094355_1064_1086_F	TCGACCTTTGGCAGGAACTAGAC	357	TRPE_AY094355_1171_1196_R	TACATCGTTTCGCCCAAGATCAATCA	885
906	TRPE_AY094355_666_688_F	GTGCATGCGGATACAGAGCAGAG	135	TRPE_AY094355_769_791_R	TTCAAAATGCGGAGGCGTATGTG	1372
907	TRPE_AY094355_757_7	TGCAAGCGCGACCACATACG	483	TRPE_AY094355_864_883_R	TGCCCAGGTACAACCTGCAT	1218

	76_F					
908	RECA_AF251469_43_68_F	TGGTACATGTGCCTTCATTGATGCTG	601	RECA_AF251469_140_163_R	TTCAAGTGCTTGCTCACCATTGTC	1375
909	RECA_AF251469_169_190_F	TGACATGCTTGTCCGTTCAGGC	446	RECA_AF251469_277_300_R	TGGCTCATAAGACGCGCTTGTAGA	1280
910	PARC_X95819_87_110_F	TGGTGACTCGGCATGTTATGAAGC	609	PARC_X95819_201_222_R	TTCGGTATAACGCATCGCAGCA	1387
911	PARC_X95819_87_110_F	TGGTGACTCGGCATGTTATGAAGC	609	PARC_X95819_192_219_R	GGTATAACGCATCGCAGCAAAAGATTTA	836
912	PARC_X95819_123_147_F	GGCTCAGCCATTTAGTTACCGCTAT	120	PARC_X95819_232_260_R	TCGCTCAGCAATAATTCACTATAAGCCGA	1081
913	PARC_X95819_43_63_F	TCAGCGCGTACAGTGGGTGAT	277	PARC_X95819_143_170_R	TTCCCCTGACCTTCGATTAAAGGATAGC	1383
914	OMPA_AY485227_272_301_F	TTACTCCATTATTGCTTGGTTACACTTTCC	655	OMPA_AY485227_364_388_R	GAGCTGCGCCAACGAATAAATCGTC	812
915	OMPA_AY485227_379_401_F	TGCGCAGCTCTTGGTATCGAGTT	509	OMPA_AY485227_492_519_R	TGCCGTAACATAGAAGTTACCGTTGATT	1223
916	OMPA_AY485227_311_335_F	TACACAACAATGGCGGTAAAGATGG	178	OMPA_AY485227_424_453_R	TACGTCGCCTTTAACTTGGTTATATTCAGC	901
917	OMPA_AY485227_415_441_F	TGCCTCGAAGCTGAATATAACCAAGTT	506	OMPA_AY485227_514_546_R	TCGGGCGTAGTTTTTAGTAATTAAATCAGAAGT	1092
918	OMPA_AY485227_494_520_F	TCAACGGTAACTTCTATGTTACTTCTG	252	OMPA_AY485227_569_596_R	TCGTCGTATTTATAGTGACCAGCACCTA	1108
919	OMPA_AY485227_551_577_F	TCAAGCCGTACGTATTATTAGGTGCTG	257	OMPA_AY485227_658_680_R	TTTAAGCGCCAGAAAGCACCAAC	1425
920	OMPA_AY485227_555_581_F	TCCGTACGTATTATTAGGTGCTGGTCA	328	OMPA_AY485227_635_662_R	TCAACACCAGCGTTACCTAAAGTACCTT	954
921	OMPA_AY485227_556_583_F	TCGTACGTATTATTAGGTGCTGGTCACT	379	OMPA_AY485227_659_683_R	TCGTTTAAGCGCCAGAAAGCACCAA	1114
922	OMPA_AY485227_657_679_F	TGTTGGTGCTTTCTGGCGCTTAA	645	OMPA_AY485227_739_765_R	TAAGCCAGCAAGAGCTGTATAGTTCCA	871
923	OMPA_AY485227_660_683_F	TGGTGCTTTCTGGCGCTTAAACGA	613	OMPA_AY485227_786_807_R	TACAGGAGCAGCAGGCTTCAAG	884
924	GYRA_AF100557_4_23_F	TCTGCCCGTGTCGTTGGTGA	402	GYRA_AF100557_119_142_R	TCGAACCGAAGTTACCCTGACCAT	1063
925	GYRA_AF100557_70_94_F	TCCATTGTTCGTATGGCTCAAGACT	316	GYRA_AF100557_178_201_R	TGCCAGCTTAGTCATACGGACTTC	1211
926	GYRB_AB008700_19_40_F	TCAGGTGGCTTACACGGCGTAG	289	GYRB_AB008700_111_140_R	TATTGCGGATCACCATGATGATATTCTTGC	941
927	GYRB_AB008700_265_292_F	TCTTTCTTGAATGCTGGTGTACGTATCG	420	GYRB_AB008700_369_395_R	TCGTTGAGATGGTTTTTACCTTCGTTG	1113
928	GYRB_AB008700_368_394_F	TCAACGAAGGTAAAAACCATCTCAACG	251	GYRB_AB008700_466_494_R	TTTGTGAAACAGCGAACATTTTCTTGGTA	1440
929	GYRB_AB008700_477_504_F	TGTTCGCTGTTTCACAAACAACATTCCA	641	GYRB_AB008700_611_632_R	TCACGCGCATCATCACCAGTCA	977
930	GYRB_AB008700_760_7	TACTTACTTGAGAATCCACAAGCTGC	198	GYRB_AB008700_862_888_R	ACCTGCAATATCTAATGCACTCTTACG	729

	87_F	AA				
931	WAAA_Z96925_2_29_F	TCTTGCTCTTTCGTGAGTTCAGTAAATG	416	WAAA_Z96925_115_138_R	CAAGCGGTTTGCCTCAAATAGTCA	758
932	WAAA_Z96925_286_311_F	TCGATCTGGTTTCATGCTGTTTCAGT	360	WAAA_Z96925_394_412_R	TGGCACGAGCCTGACCTGT	1274
939	RPOB_EC_3798_3821_F	TGGGCAGCGTTTCGGCGAAATGGA	581	RPOB_EC_3862_3889_R	TGTCCGACTTGACGGTCAGCATTTCCTG	1326
940	RPOB_EC_3798_3821_F	TGGGCAGCGTTTCGGCGAAATGGA	581	RPOB_EC_3862_3889_2_R	TGTCCGACTTGACGGTTAGCATTTCCTG	1327
941	TUFB_EC_275_299_F	TGATCACTGGTGCTGCTCAGATGGA	468	TUFB_EC_337_362_R	TGGATGTGCTCACGAGTCTGTGGCAT	1271
942	TUFB_EC_251_278_F	TGCACGCCGACTATGTTAAGAACATGAT	493	TUFB_EC_337_360_R	TATGTGCTCACGAGTTTGCGGCAT	937
949	GYRB_AB008700_760_787_F	TACTTACTTGAGAATCCACAAGCTGCAA	198	GYRB_AB008700_862_888_2_R	TCCTGCAATATCTAATGCACTCTTACG	1050
958	RPOC_EC_2223_2243_F	TGGTATGCGTGGTCTGATGGC	605	RPOC_EC_2329_2352_R	TGCTAGACCTTTACGTGCACCGTG	1243
959	RPOC_EC_918_938_F	TCTGGATAACGGTCGTCGCGG	404	RPOC_EC_1009_1031_R	TCCAGCAGGTTCTGACGGAAACG	1004
960	RPOC_EC_2334_2357_F	TGCTCGTAAGGGTCTGGCGGATAC	523	RPOC_EC_2380_2403_R	TACTAGACGACGGGTCAGGTAACC	905
961	RPOC_EC_917_938_F	TATTGGACAACGGTCGTCGCGG	242	RPOC_EC_1009_1034_R	TTACCGAGCAGGTTCTGACGGAAACG	1362
962	RPOB_EC_2005_2027_F	TCGTTCCTGGAACACGATGACGC	387	RPOB_EC_2041_2064_R	TTGACGTTGCATGTTCGAGCCCAT	1399
963	RPOB_EC_1527_1549_F	TCAGCTGTCGCAGTTCATGGACC	282	RPOB_EC_1630_1649_R	TCGTCGCGGACTTCGAAGCC	1104
964	INFB_EC_1347_1367_F	TGCGTTTACCGCAATGCGTGC	515	INFB_EC_1414_1432_R	TCGGCATCACGCCGTCGTC	1090
965	VALS_EC_1128_1151_F	TATGCTGACCGACCAGTGGTACGT	237	VALS_EC_1231_1257_R	TTCGCGCATCCAGGAGAAGTACATGTT	1384
978	RPOC_EC_2145_2175_F	TCAGGAGTCGTTCAACTCGATCTACATGATG	285	RPOC_EC_2228_2247_R	TTACGCCATCAGGCCACGCA	1363
1045	CJST_CJ_1668_1700_F	TGCTCGAGTGATTGACTTTGCTAAATTTAGAGA	522	CJST_CJ_1774_1799_R	TGAGCGTGTGGAAAAGGACTTGGATG	1170
1046	CJST_CJ_2171_2197_F	TCGTTTGGTGGTGGTAGATGAAAAAGG	388	CJST_CJ_2283_2313_R	TCTCTTTCAAAGCACCATTGCTCATTATAGT	1126
1047	CJST_CJ_584_616_F	TCCAGGACAAATGTATGAAAAATGTCCAAGAAG	315	CJST_CJ_663_692_R	TTCATTTTCTGGTCCAAAGTAAGCAGTATC	1379
1048	CJST_CJ_360_394_F	TCCTGTTATCCCTGAAGTAGTTAATCAAGTTTGTT	346	CJST_CJ_442_476_R	TCAACTGGTTCAAAAACATTAAGTTGTAATTGTCC	955
1049	CJST_CJ_2636_2668_F	TGCCTAGAAGATCTTAAAAATTTCCGCCAACTT	504	CJST_CJ_2753_2777_R	TTGCTGCCATAGCAAAGCCTACAGC	1409
1050	CJST_CJ_1290_1320_F	TGGCTTATCCAAATTTAGATCGTGGTTTTAC	575	CJST_CJ_1406_1433_R	TTTGCTCATGATCTGCATGAAGCATAAA	1437
1051	CJST_CJ_3267_3293_F	TTTGATTTTACGCCGTCCTCCAGGTCG	707	CJST_CJ_3356_3385_R	TCAAAGAACCCGCACCTAATTCATCATTTA	951
1052	CJST_CJ_5_39_F	TAGGCGAAGATATACAAAGAGTATTAGAAGCTAGA	222	CJST_CJ_104_137_R	TCCCTTATTTTTCTTTCTACTACCTTCGGATAAT	1029
1053	CJST_CJ_1080_1110_F	TTGAGGGTATGCACCGTCTTTTTGATTCTTT	681	CJST_CJ_1166_1198_R	TCCCCTCATGTTTAAATGATCAGGATAAAAAGC	1022
1054	CJST_CJ_2060_2090_F	TCCCGGACTTAATATCAATGAAAATTGTGGA	323	CJST_CJ_2148_2174_R	TCGATCCGCATCACCATCAAAAGCAAA	1068

1055	CJST_CJ_2869_2895_F	TGAAGCTTGTTCTTTAGCAGGACTTCA	432	CJST_CJ_2979_3007_R	TCCTCCTTGTGCCTCAAAACGCATTTTTA	1045
1056	CJST_CJ_1880_1910_F	TCCCAATTAATTCTGCCATTTTTCCAGGTAT	317	CJST_CJ_1981_2011_R	TGGTTCTTACTTGCTTTGCATAAACTTTCCA	1309
1057	CJST_CJ_2185_2212_F	TAGATGAAAAGGGCGAAGTGGCTAATGG	208	CJST_CJ_2283_2316_R	TGAATTCTTTCAAAGCACCATTGCTCATTATAGT	1152
1058	CJST_CJ_1643_1670_F	TTATCGTTTGTGGAGCTAGTGCTTATGC	660	CJST_CJ_1724_1752_R	TGCAATGTGTGCTATGTCAGCAAAAAGAT	1198
1059	CJST_CJ_2165_2194_F	TGCGGATCGTTTGGTGGTTGTAGATGAAAA	511	CJST_CJ_2247_2278_R	TCCACACTGGATTGTAATTTACCTTGTTCTTT	1002
1060	CJST_CJ_599_632_F	TGAAAAATGTCCAAGAAGCATAGCAAAAAAAGCA	424	CJST_CJ_711_743_R	TCCCGAACAATGAGTTGTATCAACTATTTTTAC	1024
1061	CJST_CJ_360_393_F	TCCTGTTATCCCTGAAGTAGTTAATCAAGTTTGT	345	CJST_CJ_443_477_R	TACAACTGGTTCAAAAACATTAAGCTGTAATTGTC	882
1062	CJST_CJ_2678_2703_F	TCCCCAGGACACCCTGAAATTTCAAC	321	CJST_CJ_2760_2787_R	TGTGCTTTTTTTGCTGCCATAGCAAAGC	1339
1063	CJST_CJ_1268_1299_F	AGTTATAAACACGGCTTTCCTATGGCTTATCC	29	CJST_CJ_1349_1379_R	TCGGTTTAAGCTCTACATGATCGTAAGGATA	1096
1064	CJST_CJ_1680_1713_F	TGATTTTGCTAAATTTAGAGAAATTGCGGATGAA	479	CJST_CJ_1795_1822_R	TATGTGTAGTTGAGCTTACTACATGAGC	938
1065	CJST_CJ_2857_2887_F	TGGCATTTCTTATGAAGCTTGTTCTTTAGCA	565	CJST_CJ_2965_2998_R	TGCTTCAAAACGCATTTTTACATTTTCGTTAAAG	1253
1070	RNASEP_BKM_580_599_F	TGCGGGTAGGGAGCTTGAGC	512	RNASEP_BKM_665_686_R	TCCGATAAGCCGGATTCTGTGC	1034
1071	RNASEP_BKM_616_637_F	TCCTAGAGGAATGGCTGCCACG	333	RNASEP_BKM_665_687_R	TGCCGATAAGCCGGATTCTGTGC	1222
1072	RNASEP_BDP_574_592_F	TGGCACGGCCATCTCCGTG	561	RNASEP_BDP_616_635_R	TCGTTTCACCCTGTCATGCCG	1115
1073	23S_BRM_1110_1129_F	TGCGCGGAAGATGTAACGGG	510	23S_BRM_1176_1201_R	TCGCAGGCTTACAGAACGCTCTCCTA	1074
1074	23S_BRM_515_536_F	TGCATACAAACAGTCGGAGCCT	496	23S_BRM_616_635_R	TCGGACTCGCTTTCGCTACG	1088
1075	RNASEP_CLB_459_487_F	TAAGGATAGTGCAACAGAGATATACCGCC	162	RNASEP_CLB_498_526_R	TGCTCTTACCTCACCGTTCCACCCTTACC	1247
1076	RNASEP_CLB_459_487_F	TAAGGATAGTGCAACAGAGATATACCGCC	162	RNASEP_CLB_498_522_R	TTTACCTCGCCTTTCCACCCTTACC	1426
1077	ICD_CXB_93_120_F	TCCTGACCGACCCATTATTCCCTTTATC	343	ICD_CXB_172_194_R	TAGGATTTTTCCACGGCGGCATC	921
1078	ICD_CXB_92_120_F	TTCCTGACCGACCCATTATTCCCTTTATC	671	ICD_CXB_172_194_R	TAGGATTTTTCCACGGCGGCATC	921
1079	ICD_CXB_176_198_F	TCGCCGTGGAAAAATCCTACGCT	369	ICD_CXB_224_247_R	TAGCCTTTTCTCCGGCGTAGATCT	916
1080	IS1111A_NC002971_6866_6891_F	TCAGTATGTATCCACCGTAGCCAGTC	290	IS1111A_NC002971_6928_6954_R	TAAACGTCCGATACCAATGGTTCGCTC	848
1081	IS1111A_NC002971_7456_7483_F	TGGGTGACATTCATCAATTTCATCGTTC	594	IS1111A_NC002971_7529_7554_R	TCAACAACACCTCCTTATTCCCACTC	952
1082	RNASEP_RKP_419_448_F	TGGTAAGAGCGCACCGGTAAGTTGGTAACA	599	RNASEP_RKP_542_565_R	TCAAGCGATCTACCCGCATTACAA	957

1083	RNASEP_RKP_422_443_F	TAAGAGCGCACCGGTAAGTTGG	159	RNASEP_RKP_542_565_R	TCAAGCGATCTACCCGCATTACAA	957
1084	RNASEP_RKP_466_491_F	TCCACCAAGAGCAAGATCAAATAGGC	310	RNASEP_RKP_542_565_R	TCAAGCGATCTACCCGCATTACAA	957
1085	RNASEP_RKP_264_287_F	TCTAAATGGTCGTGCAGTTGCGTG	391	RNASEP_RKP_295_321_R	TCTATAGAGTCCGGACTTTCCTCGTGA	1119
1086	RNASEP_RKP_426_448_F	TGCATACCGGTAAGTTGGCAACA	497	RNASEP_RKP_542_565_R	TCAAGCGATCTACCCGCATTACAA	957
1087	OMPB_RKP_860_890_F	TTACAGGAAGTTTAGGTGGTAATCTAAAAGG	654	OMPB_RKP_972_996_R	TCCTGCAGCTCTACCTGCTCCATTA	1051
1088	OMPB_RKP_1192_1221_F	TCTACTGATTTTGGTAATCTTGCAGCACAG	392	OMPB_RKP_1288_1315_R	TAGCAgCAAAAGTTATCACACCTGCAGT	910
1089	OMPB_RKP_3417_3440_F	TGCAAGTGGTACTTCAACATGGGG	485	OMPB_RKP_3520_3550_R	TGGTTGTAGTTCCTGTAGTTGTTGCATTAAC	1310
1090	GLTA_RKP_1043_1072_F	TGGGACTTGAAGCTATCGCTCTTAAAGATG	576	GLTA_RKP_1138_1162_R	TGAACATTTGCGACGGTATACCCAT	1147
1091	GLTA_RKP_400_428_F	TCTTCTCATCCTATGGCTATTATGCTTGC	413	GLTA_RKP_499_529_R	TGGTGGGTATCTTAGCAATCATTCTAATAGC	1305
1092	GLTA_RKP_1023_1055_F	TCCGTTCTTACAAATAGCAATAGAACTTGAAGC	330	GLTA_RKP_1129_1156_R	TTGGCGACGGTATACCCATAGCTTTATA	1415
1093	GLTA_RKP_1043_1072_2_F	TGGAGCTTGAAGCTATCGCTCTTAAAGATG	553	GLTA_RKP_1138_1162_R	TGAACATTTGCGACGGTATACCCAT	1147
1094	GLTA_RKP_1043_1072_3_F	TGGAACTTGAAGCTCTCGCTCTTAAAGATG	543	GLTA_RKP_1138_1164_R	TGTGAACATTTGCGACGGTATACCCAT	1330
1095	GLTA_RKP_400_428_F	TCTTCTCATCCTATGGCTATTATGCTTGC	413	GLTA_RKP_505_534_R	TGCGATGGTAGGTATCTTAGCAATCATTCT	1230
1096	CTXA_VBC_117_142_F	TCTTATGCCAAGAGGACAGAGTGAGT	410	CTXA_VBC_194_218_R	TGCCTAACAAATCCCGTCTGAGTTC	1226
1097	CTXA_VBC_351_377_F	TGTATTAGGGGCATACAGTCCTCATCC	630	CTXA_VBC_441_466_R	TGTCATCAAGCACCCCAAAATGAACT	1324
1098	RNASEP_VBC_331_349_F	TCCGCGGAGTTGACTGGGT	325	RNASEP_VBC_388_414_R	TGACTTTCCTCCCCCTTATCAGTCTCC	1163
1099	TOXR_VBC_135_158_F	TCGATTAGGCAGCAACGAAAGCCG	362	TOXR_VBC_221_246_R	TTCAAAACCTTGCTCTCGCCAAACAA	1370
1100	ASD_FRT_1_29_F	TTGCTTAAAGTTGGTTTTATTGGTTGGCG	690	ASD_FRT_86_116_R	TGAGATGTCGAAAAAAACGTTGGCAAAATAC	1164
1101	ASD_FRT_43_76_F	TCAGTTTTAATGTCTCGTATGATCGAATCAAAAG	295	ASD_FRT_129_156_R	TCCATATTGTTGCATAAAACCTGTTGGC	1009
1102	GALE_FRT_168_199_F	TTATCAGCTAGACCTTTTAGGTAAAGCTAAGC	658	GALE_FRT_241_269_R	TCACCTACAGCTTTAAAGCCAGCAAAATG	973
1103	GALE_FRT_834_865_F	TCAAAAAGCCCTAGGTAAAGAGATTCCATATC	245	GALE_FRT_901_925_R	TAGCCTTGGCAACATCAGCAAAACT	915
1104	GALE_FRT_308_339_F	TCCAAGGTACACTAAACTTACTTGAGCTAATG	306	GALE_FRT_390_422_R	TCTTCTGTAAAGGGTGGTTTATTATTCATCCCA	1136
1105	IPAH_SGF_258_277_F	TGAGGACCGTGTCGCGCTCA	458	IPAH_SGF_301_327_R	TCCTTCTGATGCCTGATGGACCAGGAG	1055
1106	IPAH_SGF_113_134_F	TCCTTGACCGCCTTTCCGATAC	350	IPAH_SGF_172_191_R	TTTTCCAGCCATGCAGCGAC	1441

1107	IPAH_SGF_462_486_F	TCAGACCATGCTCGCAGAGAAACTT	271	IPAH_SGF_522_540_R	TGTCACTCCCGACACGCCA	1322
1111	RNASEP_BRM_461_488_F	TAAACCCCATCGGGAGCAAGACCGAATA	147	RNASEP_BRM_542_561_R	TGCCTCGCGCAACCTACCCG	1227
1112	RNASEP_BRM_325_347_F	TACCCCAGGGAAAGTGCCACAGA	185	RNASEP_BRM_402_428_R	TCTCTTACCCCACCCTTTCACCCTTAC	1125
1128	HUPB_CJ_113_134_F	TAGTTGCTCAAACAGCTGGGCT	230	HUPB_CJ_157_188_R	TCCCTAATAGTAGAAATAACTGCATCAGTAGC	1028
1129	HUPB_CJ_76_102_F	TCCCGGAGCTTTTATGACTAAAGCAGAT	324	HUPB_CJ_157_188_R	TCCCTAATAGTAGAAATAACTGCATCAGTAGC	1028
1130	HUPB_CJ_76_102_F	TCCCGGAGCTTTTATGACTAAAGCAGAT	324	HUPB_CJ_114_135_R	TAGCCCAGCTGTTTGAGCAACT	913
1151	AB_MLST-11-OIF007_62_91_F	TGAGATTGCTGAACATTTAATGCTGATTGA	454	AB_MLST-11-OIF007_169_203_R	TTGTACATTTGAAACAATATGCATGACATGTGAAT	1418
1152	AB_MLST-11-OIF007_185_214_F	TATTGTTTCAAATGTACAAGGTGAAGTGCG	243	AB_MLST-11-OIF007_291_324_R	TCACAGGTTCTACTTCATCAATAATTTCCATTGC	969
1153	AB_MLST-11-OIF007_260_289_F	TGGAACGTTATCAGGTGCCCCAAAAATTCG	541	AB_MLST-11-OIF007_364_393_R	TTGCAATCGACATATCCATTTCACCATGCC	1400
1154	AB_MLST-11-OIF007_206_239_F	TGAAGTGCGTGATGATATCGATGCACTTGATGTA	436	AB_MLST-11-OIF007_318_344_R	TCCGCCAAAAACTCCCCTTTTCACAGG	1036
1155	AB_MLST-11-OIF007_522_552_F	TCGGTTTAGTAAAAGAACGTATTGCTCAACC	378	AB_MLST-11-OIF007_587_610_R	TTCTGCTTGAGGAATAGTGCGTGG	1392
1156	AB_MLST-11-OIF007_547_571_F	TCAACCTGACTGCGTGAATGGTTGT	250	AB_MLST-11-OIF007_656_686_R	TACGTTCTACGATTTCTTCATCAGGTACATC	902
1157	AB_MLST-11-OIF007_601_627_F	TCAAGCAGAAGCTTTGGAAGAAGAAGG	256	AB_MLST-11-OIF007_710_736_R	TACAACGTGATAAACACGACCAGAAGC	881
1158	AB_MLST-11-OIF007_1202_1225_F	TCGTGCCCGCAATTTGCATAAAGC	384	AB_MLST-11-OIF007_1266_1296_R	TAATGCCGGGTAGTGCAATCCATTCTTCTAG	878
1159	AB_MLST-11-OIF007_1202_1225_F	TCGTGCCCGCAATTTGCATAAAGC	384	AB_MLST-11-OIF007_1299_1316_R	TGCACCTGCGGTCGAGCG	1199
1160	AB_MLST-11-OIF007_1234_1264_F	TTGTAGCACAGCAAGGCAAATTTCCTGAAAC	694	AB_MLST-11-OIF007_1335_1362_R	TGCCATCCATAATCACGCCATACTGACG	1215
1161	AB_MLST-11-OIF007_1327_1356_F	TAGGTTTACGTCAGTATGGCGTGATTATGG	225	AB_MLST-11-OIF007_1422_1448_R	TGCCAGTTTCCACATTTCACGTTCGTG	1212
1162	AB_MLST-11-OIF007_1345_1369_F	TCGTGATTATGGATGGCAACGTGAA	383	AB_MLST-11-OIF007_1470_1494_R	TCGCTTGAGTGTAGTCATGATTGCG	1083
1163	AB_MLST-11-OIF007_1351_1375_F	TTATGGATGGCAACGTGAAACGCGT	662	AB_MLST-11-OIF007_1470_1494_R	TCGCTTGAGTGTAGTCATGATTGCG	1083
1164	AB_MLST-11-OIF007_1387_1412_F	TCTTTGCCATTGAAGATGACTTAAGC	422	AB_MLST-11-OIF007_1470_1494_R	TCGCTTGAGTGTAGTCATGATTGCG	1083
1165	AB_MLST-11-OIF007_1542_1569_F	TACTAGCGGTAAGCTTAAACAAGATTGC	194	AB_MLST-11-OIF007_1656_1680_R	TGAGTCGGGTTCACTTTACCTGGCA	1173
1166	AB_MLST-11-OIF007_1566_1593_F	TTGCCAATGATATTCGTTGGTTAGCAAG	684	AB_MLST-11-OIF007_1656_1680_R	TGAGTCGGGTTCACTTTACCTGGCA	1173
1167	AB_MLST-11-OIF007_1611_1638_F	TCGGCGAAATCCGTATTCCTGAAAATGA	375	AB_MLST-11-OIF007_1731_1757_R	TACCGGAAGCACCAGCGACATTAATAG	890

1168	AB_MLST-11-OIF007_1726_1752_F	TACCACTATTAATGTCGCTGGTGCTTC	182	AB_MLST-11-OIF007_1790_1821_R	TGCAACTGAATAGATTGCAGTAAGTTATAAGC	1195
1169	AB_MLST-11-OIF007_1792_1826_F	TTATAACTTACTGCAATCTATTCAGTTGCTTGGTG	656	AB_MLST-11-OIF007_1876_1909_R	TGAATTATGCAAGAAGTGATCAATTTTCTCACGA	1151
1170	AB_MLST-11-OIF007_1792_1826_F	TTATAACTTACTGCAATCTATTCAGTTGCTTGGTG	656	AB_MLST-11-OIF007_1895_1927_R	TGCCGTAACTAACATAAGAGAATTATGCAAGAA	1224
1171	AB_MLST-11-OIF007_1970_2002_F	TGGTTATGTACCAAATACTTTGTCTGAAGATGG	618	AB_MLST-11-OIF007_2097_2118_R	TGACGGCATCGATACCACCGTC	1157
1172	RNASEP_BRM_461_488_F	TAAACCCCATCGGGAGCAAGACCGAATA	147	RNASEP_BRM_542_561_2_R	TGCCTCGTGCAACCCACCCG	1228
2000	CTXB_NC002505_46_70_F	TCAGCGTATGCACATGGAACTCCTC	278	CTXB_NC002505_132_162_R	TCCGGCTAGAGATTCTGTATACGACAATATC	1039
2001	FUR_NC002505_87_113_F	TGAGTGCCAACATATCAGTGCTGAAGA	465	FUR_NC002505_205_228_R	TCCGCCTTCAAAATGGTGGCGAGT	1037
2002	FUR_NC002505_87_113_F	TGAGTGCCAACATATCAGTGCTGAAGA	465	FUR_NC002505_178_205_R	TCACGATACCTGCATCATCAAATTGGTT	974
2003	GAPA_NC002505_533_560_F	TCGACAACACCATTATCTATGGTGTGAA	356	GAPA_NC002505_646_671_R	TCAGAATCGATGCCAAATGCGTCATC	980
2004	GAPA_NC002505_694_721_F	TCAATGAACGACCAACAAGTGATTGATG	259	GAPA_NC002505_769_798_R	TCCTCTATGCAACTTAGTATCAACAGGAAT	1046
2005	GAPA_NC002505_753_782_F	TGCTAGTCAATCTATCATTCCGGTTGATAC	517	GAPA_NC002505_856_881_R	TCCATCGCAGTCACGTTTACTGTTGG	1011
2006	GYRB_NC002505_2_32_F	TGCCGGACAATTACGATTCATCGAGTATTAA	501	GYRB_NC002505_109_134_R	TCCACCACCTCAAAGACCATGTGGTG	1003
2007	GYRB_NC002505_123_152_F	TGAGGTGGTGGATAACTCAATTGATGAAGC	460	GYRB_NC002505_199_225_R	TCCGTCATCGCTGACAGAAACTGAGTT	1042
2008	GYRB_NC002505_768_794_F	TATGCAGTGGAACGATGGTTTCCAAGA	236	GYRB_NC002505_832_860_R	TGGAAACCGGCTAAGTGAGTACCACCATC	1262
2009	GYRB_NC002505_837_860_F	TGGTACTCACTTAGCGGGTTTCCG	603	GYRB_NC002505_937_957_R	TCCTTCACGCGCATCATCACC	1054
2010	GYRB_NC002505_934_956_F	TCGGGTGATGATGCGCGTGAAGG	377	GYRB_NC002505_982_1007_R	TGGCTTGAGAATTTAGGATCCGGCAC	1283
2011	GYRB_NC002505_1161_1190_F	TAAAGCCCGTGAAATGACTCGTCGTAAAGG	148	GYRB_NC002505_1255_1284_R	TGAGTCACCCTCCACAATGTATAGTTCAGA	1172
2012	OMPU_NC002505_85_110_F	TACGCTGACGGAATCAACCAAAGCGG	190	OMPU_NC002505_154_180_R	TGCTTCAGCACGGCCACCAACTTCTAG	1254
2013	OMPU_NC002505_258_283_F	TGACGGCCTATACGGTGTTGGTTTCT	451	OMPU_NC002505_346_369_R	TCCGAGACCAGCGTAGGTGTAACG	1033
2014	OMPU_NC002505_431_455_F	TCACCGATATCATGGCTTACCACGG	266	OMPU_NC002505_544_567_R	TCGGTCAGCAAAACGGTAGCTTGC	1094
2015	OMPU_NC002505_533_557_F	TAGGCGTGAAAGCAAGCTACCGTTT	223	OMPU_NC002505_625_651_R	TAGAGAGTAGCCATCTTCACCGTTGTC	908
2016	OMPU_NC002505_689_713_F	TAGGTGCTGGTTACGCAGATCAAGA	224	OMPU_NC002505_725_751_R	TGGGGTAAGACGCGGCTAGCATGTATT	1291
2017	OMPU_NC002505_727_7	TACATGCTAGCCGCGTCTTAC	181	OMPU_NC002505_811_835_R	TAGCAGCTAGCTCGTAACCAGTGTA	911

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2018	OMPU_NC002505_931_953_F	TACTACTTCAAGCCGAACTTCCG	193	OMPU_NC002505_1033_1053_R	TTAGAAGTCGTAACGTGGACC	1368
2019	OMPU_NC002505_927_953_F	TACTTACTACTTCAAGCCGAACTTCCG	197	OMPU_NC002505_1033_1054_R	TGGTTAGAAGTCGTAACGTGGACC	1307
2020	TCPA_NC002505_48_73_F	TCACGATAAGAAAACCGGTCAAGAGG	269	TCPA_NC002505_148_170_R	TTCTGCGAATCAATCGCACGCTG	1391
2021	TDH_NC004605_265_289_F	TGGCTGACATCCTACATGACTGTGA	574	TDH_NC004605_357_386_R	TGTTGAAGCTGTACTTGACCTGATTTTACG	1351
2022	VVHA_NC004460_772_802_F	TCTTATTCCAACTTCAAACCGAACTATGACG	412	VVHA_NC004460_862_886_R	TACCAAAGCGTGCACGATAGTTGAG	887
2023	23S_EC_2643_2667_F	TGCCTGTTCTTAGTACGAGAGGACC	508	23S_EC_2746_2770_R	TGGGTTTCGCGCTTAGATGCTTTCA	1297
2024	16S_EC_713_732_TMOD_F	TAGAACACCGATGGCGAAGGC	202	16S_EC_789_811_R	TGCGTGGACTACCAGGGTATCTA	1240
2025	16S_EC_784_806_F	TGGATTAGAGACCCTGGTAGTCC	560	16S_EC_880_897_TMOD_R	TGGCCGTACTCCCCAGGCG	1278
2026	16S_EC_959_981_F	TGTCGATGCAACGCGAAGAACCT	634	16S_EC_1052_1074_R	TACGAGCTGACGACAGCCATGCA	896
2027	TUFB_EC_956_979_F	TGCACACGCCGTTCTTCAACAACT	489	TUFB_EC_1034_1058_2_R	TGCATCACCATTTCCTTGTCCTTCG	1204
2028	RPOC_EC_2146_2174_TMOD_F	TCAGGAGTCGTTCAACTCGATCTACATGAT	284	RPOC_EC_2227_2249_R	TGCTAGGCCATCAGGCCACGCAT	1244
2029	RPOB_EC_1841_1866_F	TGGTTATCGCTCAGGCGAACTCCAAC	617	RPOB_EC_1909_1929_TMOD_R	TGCTGGATTCGCCTTTGCTACG	1250
2030	RPLB_EC_650_679_TMOD_F	TGACCTACAGTAAGAGGTTCTGTAATGAACC	449	RPLB_EC_739_763_R	TGCCAAGTGCTGGTTTACCCCATGG	1208
2031	RPLB_EC_690_710_F	TCCACACGGTGGTGGTGAAGG	309	RPLB_EC_737_760_R	TGGGTGCTGGTTTACCCCATGGAG	1295
2032	INFB_EC_1366_1393_F	TCTCGTGGTGCACAAGTAACGGATATTA	397	INFB_EC_1439_1469_R	TGTGCTGCTTTCGCATGGTTAATTGCTTCAA	1335
2033	VALS_EC_1105_1124_TMOD_F	TCGTGGCGGCGTGGTTATCGA	385	VALS_EC_1195_1219_R	TGGGTACGAACTGGATGTCGCCGTT	1292
2034	SSPE_BA_113_137_F	TGCAAGCAAACGCACAATCAGAAGC	482	SSPE_BA_197_222_TMOD_R	TTGCACGTCTGTTTCAGTTGCAAATTC	1402
2035	RPOC_EC_2218_2241_TMOD_F	TCTGGCAGGTATGCGTGGTCTGATG	405	RPOC_EC_2313_2338_R	TGGCACCGTGGGTTGAGATGAAGTAC	1273
2056	MECI-R_NC003923-41798-41609_33_60_F	TTTACACATATCGTGAGCAATGAACTGA	698	MECI-R_NC003923-41798-41609_86_113_R	TTGTGATATGGAGGTGTAGAAGGTGTTA	1420
2057	AGR-III_NC003923-2108074-2109507_1_23_F	TCACCAGTTTGCCACGTATCTTCAA	263	AGR-III_NC003923-2108074-2109507_56_79_R	ACCTGCATCCCTAAACGTACTTGC	730
2058	AGR-III_NC003923-2108074-2109507_569_596_F	TGAGCTTTTAGTTGACTTTTTCAACAGC	457	AGR-III_NC003923-2108074-2109507_622_653_R	TACTTCAGCTTCGTCCAATAAAAAATCACAAT	906
2059	AGR-III_NC003923-2108074-2109507_1024_1052_F	TTTCACACAGCGTGTTTATAGTTCTACCA	701	AGR-III_NC003923-2108074-2109507_1070_1098_R	TGTAGGCAAGTGCATAAGAAATTGATACA	1319
2060	AGR-I_AJ617706_622_651_	TGGTGACTTCATAATGGATGAAGTTGAAGT	610	AGR-I_AJ617706_694_726_R	TCCCCATTTAATAATTCCACCTACTATCACACT	1021

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2061	AGR-I_AJ617706_580_611_F	TGGGATTTTAAAAAACATTGGTAACATCGCAG	579	AGR-I_AJ617706_626_655_R	TGGTACTTCAACTTCATCCATTATGAAGTC	1302
2062	AGR-II_NC002745-2079448-2080879_620_651_F	TCTTGCAGCAGTTTATTTGATGAACCTAAAGT	415	AGR-II_NC002745-2079448-2080879_700_731_R	TTGTTTATTGTTTCCATATGCTACACACTTTC	1424
2063	AGR-II_NC002745-2079448-2080879_649_679_F	TGTACCCGCTGAATTAACGAATTTATACGAC	624	AGR-II_NC002745-2079448-2080879_715_745_R	TCGCCATAGCTAAGTTGTTTATTGTTTCCAT	1077
2064	AGR-IV_AJ617711_931_961_F	TGGTATTCTATTTTGCTGATAATGACCTCGC	606	AGR-IV_AJ617711_1004_1035_R	TGCGCTATCAACGATTTTGACAATATATGTGA	1233
2065	AGR-IV_AJ617711_250_283_F	TGGCACTCTTGCCTTTAATATTAGTAAACTATCA	562	AGR-IV_AJ617711_309_335_R	TCCCATACCTATGGCGATAACTGTCAT	1017
2066	BLAZ_NC002952(1913827..1914672)_68_68_F	TCCACTTATCGCAAATGGAAAATTAAGCAA	312	BLAZ_NC002952(1913827..1914672)_68_68_R	TGGCCACTTTTATCAGCAACCTTACAGTC	1277
2067	BLAZ_NC002952(1913827..1914672)_68_68_2_F	TGCACTTATCGCAAATGGAAAATTAAGCAA	494	BLAZ_NC002952(1913827..1914672)_68_68_2_R	TAGTCTTTTGGAACACCGTCTTTAATTAAAGT	926
2068	BLAZ_NC002952(1913827..1914672)_68_68_3_F	TGATACTTCAACGCCTGCTGCTTTC	467	BLAZ_NC002952(1913827..1914672)_68_68_3_R	TGGAACACCGTCTTTAATTAAAGTATCTCC	1263
2069	BLAZ_NC002952(1913827..1914672)_68_68_4_F	TATACTTCAACGCCTGCTGCTTTC	232	BLAZ_NC002952(1913827..1914672)_68_68_4_R	TCTTTTCTTTGCTTAATTTTCCATTTGCGAT	1145
2070	BLAZ_NC002952(1913827..1914672)_1_33_F	TGCAATTGCTTTAGTTTTAAGTGCATGTAATTC	487	BLAZ_NC002952(1913827..1914672)_34_67_R	TTACTTCCTTACCACTTTTAGTATCTAAAGCATA	1366
2071	BLAZ_NC002952(1913827..1914672)_3_34_F	TCCTTGCTTTAGTTTTAAGTGCATGTAATTCAA	351	BLAZ_NC002952(1913827..1914672)_40_68_R	TGGGGACTTCCTTACCACTTTTAGTATCTAA	1289
2072	BSA-A_NC003923-1304065-1303589_99_125_F	TAGCGAATGTGGCTTTACTTCACAATT	214	BSA-A_NC003923-1304065-1303589_165_193_R	TGCAAGGGAAACCTAGAATTACAAACCCT	1197
2073	BSA-A_NC003923-1304065-1303589_194_218_F	ATCAATTTGGTGGCCAAGAACCTGG	32	BSA-A_NC003923-1304065-1303589_253_278_R	TGCATAGGGAAGGTAACACCATAGTT	1203
2074	BSA-A_NC003923-1304065-1303589_328_349_F	TTGACTGCGGCACAACACGGAT	679	BSA-A_NC003923-1304065-1303589_388_415_R	TAACAACGTTACCTTCGCGATCCACTAA	856
2075	BSA-A_NC003923-1304065-1303589_253_278_F	TGCTATGGTGTTACCTTCCCTATGCA	519	BSA-A_NC003923-1304065-1303589_317_344_R	TGTTGTGCCGCAGTCAAATATCTAAATA	1353
2076	BSA-B_NC003923-	TAGCAACAAATATATCTGAAGCAGCG	209	BSA-B_NC003923-1917149-	TGTGAAGAACTTTCAAATCTGTGAATCC	1331

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	1917149-1914156_953_982_F	TACT		1914156_1011_1039_R	A	
2077	BSA-B_NC003923-1917149-1914156_1050_1081_F	TGAAAAGTATGGATTTGAACAACTCGTGAATA	426	BSA-B_NC003923-1917149-1914156_1109_1136_R	TCTTCTTGAAAAATTGTTGTCCCGAAAC	1138
2078	BSA-B_NC003923-1917149-1914156_1260_1286_F	TCATTATCATGCGCCAATGAGTGCAGA	300	BSA-B_NC003923-1917149-1914156_1323_1353_R	TGGACTAATAACAATGAGCTCATTGTACTGA	1267
2079	BSA-B_NC003923-1917149-1914156_2126_2153_F	TTTCATCTTATCGAGGACCCGAAATCGA	703	BSA-B_NC003923-1917149-1914156_2186_2216_R	TGAATATGTAATGCAAACCAGTCTTTGTCAT	1148
2080	ERMA_NC002952-55890-56621_366_392_F	TCGCTATCTTATCGTTGAGAAGGGATT	372	ERMA_NC002952-55890-56621_487_513_R	TGAGTCTACACTTGGCTTAGGATGAAA	1174
2081	ERMA_NC002952-55890-56621_366_395_F	TAGCTATCTTATCGTTGAGAAGGGATTTGC	217	ERMA_NC002952-55890-56621_438_465_R	TGAGCATTTTTATATCCATCTCCACCAT	1167
2082	ERMA_NC002952-55890-56621_374_402_F	TGATCGTTGAGAAGGGATTTGCGAAAAGA	470	ERMA_NC002952-55890-56621_473_504_R	TCTTGGCTTAGGATGAAAATATAGTGGTGGTA	1143
2083	ERMA_NC002952-55890-56621_404_427_F	TGCAAAATCTGCAACGAGCTTTGG	480	ERMA_NC002952-55890-56621_491_520_R	TCAATACAGAGTCTACACTTGGCTTAGGAT	964
2084	ERMA_NC002952-55890-56621_489_516_F	TCATCCTAAGCCAAGTGTAGACTCTGTA	297	ERMA_NC002952-55890-56621_586_615_R	TGGACGATATTCACGGTTTACCCACTTATA	1266
2085	ERMA_NC002952-55890-56621_586_614_F	TATAAGTGGGTAAACCGTGAATATCGTGT	231	ERMA_NC002952-55890-56621_640_665_R	TTGACATTTGCATGCTTCAAAGCCTG	1397
2086	ERMC_NC005908-2004-2738_85_116_F	TCTGAACATGATAATATCTTTGAAATCGGCTC	399	ERMC_NC005908-2004-2738_173_206_R	TCCGTAGTTTTGCATAATTTATGGTCTATTTCAA	1041
2087	ERMC_NC005908-2004-2738_90_120_F	TCATGATAATATCTTTGAAATCGGCTCAGGA	298	ERMC_NC005908-2004-2738_160_189_R	TTTATGGTCTATTTCAATGGCAGTTACGAA	1429
2088	ERMC_NC005908-2004-2738_115_139_F	TCAGGAAAAGGGCATTTTACCCTTG	283	ERMC_NC005908-2004-2738_161_187_R	TATGGTCTATTTCAATGGCAGTTACGA	936
2089	ERMC_NC005908-2004-2738_374_397_F	TAATCGTGGAATACGGGTTTGCTA	168	ERMC_NC005908-2004-2738_425_452_R	TCAACTTCTGCCATTAAAAGTAATGCCA	956
2090	ERMC_NC005908-2004-2738_101_125_F	TCTTTGAAATCGGCTCAGGAAAAGG	421	ERMC_NC005908-2004-2738_159_188_R	TGATGGTCTATTTCAATGGCAGTTACGAAA	1185
2091	ERMB_Y13600-625-1362_291_321_F	TGTTGGGAGTATTCCTTACCATTTAAGCACA	644	ERMB_Y13600-625-1362_352_380_R	TCAACAATCAGATAGATGTCAGACGCATG	953
2092	ERMB_Y13600-625-1362_344_367_F	TGGAAAGCCATGCGTCTGACATCT	536	ERMB_Y13600-625-1362_415_437_R	TGCAAGAGCAACCCTAGTGTTCG	1196
2093	ERMB_Y13600-625-1362_404_429_F	TGGATATTCACCGAACACTAGGGTTG	556	ERMB_Y13600-625-1362_471_493_R	TAGGATGAAAGCATTCCGCTGGC	919

2094	ERMB_Y13600-625-1362_465_487_F	TAAGCTGCCAGCGGAATGCTTTC	161	ERMB_Y13600-625-1362_521_545_R	TCATCTGTGGTATGGCGGGTAAGTT	989
2095	PVLUK_NC003923-1529595-1531285_688_713_F	TGAGCTGCATCAACTGTATTGGATAG	456	PVLUK_NC003923-1529595-1531285_775_804_R	TGGAAAACTCATGAAATTAAAGTGAAAGGA	1261
2096	PVLUK_NC003923-1529595-1531285_1039_1068_F	TGGAACAAAATAGTCTCTCGGATTTTGACT	539	PVLUK_NC003923-1529595-1531285_1095_1125_R	TCATTAGGTAAAATGTCTGGACATGATCCAA	993
2097	PVLUK_NC003923-1529595-1531285_908_936_F	TGAGTAACATCCATATTTCTGCCATACGT	461	PVLUK_NC003923-1529595-1531285_950_978_R	TCTCATGAAAAAGGCTCAGGAGATACAAG	1124
2098	PVLUK_NC003923-1529595-1531285_610_633_F	TCGGAATCTGATGTTGCAGTTGTT	373	PVLUK_NC003923-1529595-1531285_654_682_R	TCACACCTGTAAGTGAGAAAAAGGTTGAT	968
2099	SA442_NC003923-2538576-2538831_11_35_F	TGTCGGTACACGATATTCTTCACGA	635	SA442_NC003923-2538576-2538831_98_124_R	TTTCCGATGCAACGTAATGAGATTTCA	1433
2100	SA442_NC003923-2538576-2538831_98_124_F	TGAAATCTCATTACGTTGCATCGGAAA	427	SA442_NC003923-2538576-2538831_163_188_R	TCGTATGACCAGCTTCGGTACTACTA	1098
2101	SA442_NC003923-2538576-2538831_103_126_F	TCTCATTACGTTGCATCGGAAACA	395	SA442_NC003923-2538576-2538831_161_187_R	TTTATGACCAGCTTCGGTACTACTAAA	1428
2102	SA442_NC003923-2538576-2538831_166_188_F	TAGTACCGAAGCTGGTCATACGA	226	SA442_NC003923-2538576-2538831_231_257_R	TGATAATGAAGGGAAACCTTTTTCACG	1179
2103	SEA_NC003923-2052219-2051456_115_135_F	TGCAGGGAACAGCTTTAGGCA	495	SEA_NC003923-2052219-2051456_173_200_R	TCGATCGTGACTCTCTTTATTTTCAGTT	1070
2104	SEA_NC003923-2052219-2051456_572_598_F	TAACTCTGATGTTTTTGATGGGAAGGT	156	SEA_NC003923-2052219-2051456_621_651_R	TGTAATTAACCGAAGGTTCTGTAGAAGTATG	1315
2105	SEA_NC003923-2052219-2051456_382_414_F	TGTATGGTGGTGTAACGTTACATGATAATAATC	629	SEA_NC003923-2052219-2051456_464_492_R	TAACCGTTTCCAAAGGTACTGTATTTTGT	861
2106	SEA_NC003923-2052219-2051456_377_406_F	TTGTATGTATGGTGGTGTAACGTTACATGA	695	SEA_NC003923-2052219-2051456_459_492_R	TAACCGTTTCCAAAGGTACTGTATTTTGTTTACC	862
2107	SEB_NC002758-2135540-2135140_208_237_F	TTTCACATGTAATTTTGATATTCGCACTGA	702	SEB_NC002758-2135540-2135140_273_298_R	TCATCTGGTTTAGGATCTGGTTGACT	988
2108	SEB_NC002758-2135540-2135140_206_235_F	TATTTCACATGTAATTTTGATATTCGCACT	244	SEB_NC002758-2135540-2135140_281_304_R	TGCAACTCATCTGGTTTAGGATCT	1194
2109	SEB_NC002758-2135540-	TAACAACTCGCCTTATGAAACGGGATATA	151	SEB_NC002758-2135540-2135140_402_402_R	TGTGCAGGCATCATGTCATACCAA	1334

	2135140_402_402_F					
2110	SEB_NC002758-2135540-2135140_402_402_2_F	TTGTATGTATGGTGGTGTAACTGAGCA	696	SEB_NC002758-2135540-2135140_402_402_2_R	TTACCATCTTCAAATACCCGAACAGTAA	1361
2111	SEC_NC003923-851678-852768_546_575_F	TTAACATGAAGGAAACCACTTTGATAATGG	648	SEC_NC003923-851678-852768_620_647_R	TGAGTTTGCACTTCAAAAGAAATTGTGT	1177
2112	SEC_NC003923-851678-852768_537_566_F	TGGAATAACAAAACATGAAGGAAACCACTT	546	SEC_NC003923-851678-852768_619_647_R	TCAGTTTGCACTTCAAAAGAAATTGTGTT	985
2113	SEC_NC003923-851678-852768_720_749_F	TGAGTTTAACAGTTCACCATATGAAACAGG	466	SEC_NC003923-851678-852768_794_815_R	TCGCCTGGTGCAGGCATCATAT	1078
2114	SEC_NC003923-851678-852768_787_810_F	TGGTATGATATGATGCCTGCACCA	604	SEC_NC003923-851678-852768_853_886_R	TCTTCACACTTTTAGAATCAACCGTTTTATTGTC	1133
2115	SED_M28521_657_682_F	TGGTGGTGAAATAGATAGGACTGCTT	615	SED_M28521_741_770_R	TGTACACCATTTATCCACAAATTGATTGGT	1318
2116	SED_M28521_690_711_F	TGGAGGTGTCACTCCACACGAA	554	SED_M28521_739_770_R	TGGGCACCATTTATCCACAAATTGATTGGTAT	1288
2117	SED_M28521_833_854_F	TTGCACAAGCAAGGCGCTATTT	683	SED_M28521_888_911_R	TCGCGCTGTATTTTTCCTCCGAGA	1079
2118	SED_M28521_962_987_F	TGGATGTTAAGGGTGATTTTCCCGAA	559	SED_M28521_1022_1048_R	TGTCAATATGAAGGTGCTCTGTGGATA	1320
2119	SEA-SEE_NC002952-2131289-2130703_16_45_F	TTTACACTACTTTTATTCATTGCCCTAACG	699	SEA-SEE_NC002952-2131289-2130703_71_98_R	TCATTTATTTCTTCGCTTTTCTCGCTAC	994
2120	SEA-SEE_NC002952-2131289-2130703_249_278_F	TGATCATCCGTGGTATAACGATTTATTAGT	469	SEA-SEE_NC002952-2131289-2130703_314_344_R	TAAGCACCATATAAGTCTACTTTTTTCCCTT	870
2121	SEE_NC002952-2131289-2130703_409_437_F	TGACATGATAATAACCGATTGACCGAAGA	445	SEE_NC002952-2131289-2130703_465_494_R	TCTATAGGTACTGTAGTTTGTTTTCCGTCT	1120
2122	SEE_NC002952-2131289-2130703_525_550_F	TGTTCAAGAGCTAGATCTTCAGGCAA	640	SEE_NC002952-2131289-2130703_586_586_R	TTTGCACCTTACCGCCAAAGCT	1436
2123	SEE_NC002952-2131289-2130703_525_549_F	TGTTCAAGAGCTAGATCTTCAGGCA	639	SEE_NC002952-2131289-2130703_586_586_2_R	TACCTTACCGCCAAAGCTGTCT	892
2124	SEE_NC002952-2131289-2130703_361_384_F	TCTGGAGGCACACCAAATAAAACA	403	SEE_NC002952-2131289-2130703_444_471_R	TCCGTCTATCCACAAGTTAATTGGTACT	1043
2125	SEG_NC002758-1955100-1954171_225_251_F	TGCTCAACCCGATCCTAAATTAGACGA	520	SEG_NC002758-1955100-1954171_321_346_R	TAACTCCTCTTCCTTCAACAGGTGGA	863

2126	SEG_NC002758-1955100-1954171_623_651_F	TGGACAATAGACAATCACTTGGATTTACA	548	SEG_NC002758-1955100-1954171_671_702_R	TGCTTTGTAATCTAGTTCCTGAATAGTAACCA	1260
2127	SEG_NC002758-1955100-1954171_540_564_F	TGGAGGTTGTTGTATGTATGGTGGT	555	SEG_NC002758-1955100-1954171_607_635_R	TGTCTATTGTCGATTGTTACCTGTACAGT	1329
2128	SEG_NC002758-1955100-1954171_694_718_F	TACAAAGCAAGACACTGGCTCACTA	173	SEG_NC002758-1955100-1954171_735_762_R	TGATTCAAATGCAGAACCATCAAACTCG	1187
2129	SEH_NC002953-60024-60977_449_472_F	TTGCAACTGCTGATTTAGCTCAGA	682	SEH_NC002953-60024-60977_547_576_R	TAGTGTTGTACCTCCATATAGACATTCAGA	927
2130	SEH_NC002953-60024-60977_408_434_F	TAGAAATCAAGGTGATAGTGGCAATGA	201	SEH_NC002953-60024-60977_450_473_R	TTCTGAGCTAAATCAGCAGTTGCA	1390
2131	SEH_NC002953-60024-60977_547_576_F	TCTGAATGTCTATATGGAGGTACAACACTA	400	SEH_NC002953-60024-60977_608_634_R	TACCATCTACCCAAACATTAGCACCAA	888
2132	SEH_NC002953-60024-60977_546_575_F	TTCTGAATGTCTATATGGAGGTACAACACT	677	SEH_NC002953-60024-60977_594_616_R	TAGCACCAATCACCCTTTCCTGT	909
2133	SEI_NC002758-1957830-1956949_324_349_F	TCAACTCGAATTTTCAACAGGTACCA	253	SEI_NC002758-1957830-1956949_419_446_R	TCACAAGGACCATTATAATCAATGCCAA	966
2134	SEI_NC002758-1957830-1956949_336_363_F	TTCAACAGGTACCAATGATTTGATCTCA	666	SEI_NC002758-1957830-1956949_420_447_R	TGTACAAGGACCATTATAATCAATGCCA	1316
2135	SEI_NC002758-1957830-1956949_356_384_F	TGATCTCAGAATCTAATAATTGGGACGAA	471	SEI_NC002758-1957830-1956949_449_474_R	TCTGGCCCCTCCATACATGTATTTAG	1129
2136	SEI_NC002758-1957830-1956949_223_253_F	TCTCAAGGTGATATTGGTGTAGGTAACTTAA	394	SEI_NC002758-1957830-1956949_290_316_R	TGGGTAGGTTTTTATCTGTGACGCCTT	1293
2137	SEJ_AF053140_1307_1332_F	TGTGGAGTAACACTGCATGAAAACAA	637	SEJ_AF053140_1381_1404_R	TCTAGCGGAACAACAGTTCTGATG	1118
2138	SEJ_AF053140_1378_1403_F	TAGCATCAGAACTGTTGTTCCGCTAG	211	SEJ_AF053140_1429_1458_R	TCCTGAAGATCTAGTTCTTGAATGGTTACT	1049
2139	SEJ_AF053140_1431_1459_F	TAACCATTCAAGAACTAGATCTTCAGGCA	153	SEJ_AF053140_1500_1531_R	TAGTCCTTTCTGAATTTTACCATCAAAGGTAC	925
2140	SEJ_AF053140_1434_1461_F	TCATTCAAGAACTAGATCTTCAGGCAAG	301	SEJ_AF053140_1521_1549_R	TCAGGTATGAAACACGATTAGTCCTTTCT	984
2141	TSST_NC002758-2137564-2138293_206_236_F	TGGTTTAGATAATTCCTTAGGATCTATGCGT	619	TSST_NC002758-2137564-2138293_278_305_R	TGTAAAAGCAGGGCTATAATAAGGACTC	1312
2142	TSST_NC002758-2137564-2138293_232_258_F	TGCGTATAAAAAACACAGATGGCAGCA	514	TSST_NC002758-2137564-2138293_289_313_R	TGCCCTTTTGTAAAAGCAGGGCTAT	1221
2143	TSST_NC002758-2137564-2138293_382_410_F	TCCAAATAAGTGGCGTTACAAATACTGAA	304	TSST_NC002758-2137564-2138293_448_478_R	TACTTTAAGGGGCTATCTTTACCATGAACCT	907

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2144	TSST_NC002758-2137564-2138293_297_325_F	TCTTTTACAAAAGGGGAAAAAGTTGACTT	423	TSST_NC002758-2137564-2138293_347_373_R	TAAGTTCCTTCGCTAGTATGTTGGCTT	874
2145	ARCC_NC003923-2725050-2724595_37_58_F	TCGCCGGCAATGCCATTGGATA	368	ARCC_NC003923-2725050-2724595_97_128_R	TGAGTTAAAATGCGATTGATTTCAGTTTCCAA	1175
2146	ARCC_NC003923-2725050-2724595_131_161_F	TGAATAGTGATAGAACTGTAGGCACAATCGT	437	ARCC_NC003923-2725050-2724595_214_245_R	TCTTCTTCTTTCGTATAAAAAGGACCAATTGG	1137
2147	ARCC_NC003923-2725050-2724595_218_249_F	TTGGTCCTTTTTATACGAAAGAAGAAGTTGAA	691	ARCC_NC003923-2725050-2724595_322_353_R	TGGTGTTCTAGTATAGATTGAGGTAGTGGTGA	1306
2148	AROE_NC003923-1674726-1674277_371_393_F	TTGCGAATAGAACGATGGCTCGT	686	AROE_NC003923-1674726-1674277_435_464_R	TCGAATTCAGCTAAATACTTTTCAGCATCT	1064
2149	AROE_NC003923-1674726-1674277_30_62_F	TGGGGCTTTAAATATTCCAATTGAAGATTTTCA	590	AROE_NC003923-1674726-1674277_155_181_R	TACCTGCATTAATCGCTTGTTCATCAA	891
2150	AROE_NC003923-1674726-1674277_204_232_F	TGATGGCAAGTGGATAGGGTATAATACAG	474	AROE_NC003923-1674726-1674277_308_335_R	TAAGCAATACCTTTACTTGCACCACCTG	869
2151	GLPF_NC003923-1296927-1297391_270_301_F	TGCACCGGCTATTAAGAATTACTTTGCCAACT	491	GLPF_NC003923-1296927-1297391_382_414_R	TGCAACAATTAATGCTCCGACAATTAAAGGATT	1193
2152	GLPF_NC003923-1296927-1297391_27_51_F	TGGATGGGGATTAGCGGTTACAATG	558	GLPF_NC003923-1296927-1297391_81_108_R	TAAAGACACCGCTGGGTTTAAATGTGCA	850
2153	GLPF_NC003923-1296927-1297391_239_260_F	TAGCTGGCGCGAAATTAGGTGT	218	GLPF_NC003923-1296927-1297391_323_359_R	TCACCGATAAATAAAATACCTAAAGTTAATGCCATTG	972
2154	GMK_NC003923-1190906-1191334_91_122_F	TACTTTTTTAAAACTAGGGATGCGTTTGAAGC	200	GMK_NC003923-1190906-1191334_166_197_R	TGATATTGAACTGGTGTACCATAATAGTTGCC	1180
2155	GMK_NC003923-1190906-1191334_240_267_F	TGAAGTAGAAGGTGCAAAGCAAGTTAGA	435	GMK_NC003923-1190906-1191334_305_333_R	TCGCTCTCTCAAGTGATCTAAACTTGGAG	1082
2156	GMK_NC003923-1190906-1191334_301_329_F	TCACCTCCAAGTTTAGATCACTTGAGAGA	268	GMK_NC003923-1190906-1191334_403_432_R	TGGGACGTAATCGTATAAATTCATCATTTC	1284
2157	PTA_NC003923-628885-629355_237_263_F	TCTTGTTTATGCTGGTAAAGCAGATGG	418	PTA_NC003923-628885-629355_314_345_R	TGGTACACCTGGTTTCGTTTTGATGATTTGTA	1301
2158	PTA_NC003923-628885-629355_141_171_F	TGAATTAGTTCAATCATTTGTTGAACGACGT	439	PTA_NC003923-628885-629355_211_239_R	TGCATTGTACCGAAGTAGTTCACATTGTT	1207

2159	PTA_NC003923-628885-629355_328_356_F	TCCAAACCAGGTGTATCAAGAACATCAGG	303	PTA_NC003923-628885-629355_393_422_R	TGTTCTGGATTGATTGCACAATCACCAAAG	1349
2160	TPI_NC003923-830671-831072_131_160_F	TGCAAGTTAAGAAAGCTGTTGCAGGTTTAT	486	TPI_NC003923-830671-831072_209_239_R	TGAGATGTTGATGATTTACCAGTTCCGATTG	1165
2161	TPI_NC003923-830671-831072_1_34_F	TCCCACGAAACAGATGAAGAAATTAACAAAAAAG	318	TPI_NC003923-830671-831072_97_129_R	TGGTACAACATCGTTAGCTTTACCACTTTCACG	1300
2162	TPI_NC003923-830671-831072_199_227_F	TCAAACTGGGCAATCGGAACTGGTAAATC	246	TPI_NC003923-830671-831072_253_286_R	TGGCAGCAATAGTTTGACGTACAAATGCACACAT	1275
2163	YQI_NC003923-378916-379431_142_167_F	TGAATTGCTGCTATGAAAGGTGGCTT	440	YQI_NC003923-378916-379431_259_284_R	TCGCCAGCTAGCACGATGTCATTTTC	1076
2164	YQI_NC003923-378916-379431_44_77_F	TACAACATATTATTAAAGAGACGGGTTTGAATCC	175	YQI_NC003923-378916-379431_120_145_R	TTCGTGCTGGATTTTGTCCTTGTCCT	1388
2165	YQI_NC003923-378916-379431_135_160_F	TCCAGCACGAATTGCTGCTATGAAAG	314	YQI_NC003923-378916-379431_193_221_R	TCCAACCCAGAACCACATACTTTATTCAC	997
2166	YQI_NC003923-378916-379431_275_300_F	TAGCTGGCGGTATGGAGAATATGTCT	219	YQI_NC003923-378916-379431_364_396_R	TCCATCTGTTAAACCATCATATACCATGCTATC	1013
2167	BLAZ_(1913827..1914672)_546_575_F	TCCACTTATCGCAAATGGAAAATTAAGCAA	312	BLAZ_(1913827..1914672)_655_683_R	TGGCCACTTTTATCAGCAACCTTACAGTC	1277
2168	BLAZ_(1913827..1914672)_546_575_2_F	TGCACTTATCGCAAATGGAAAATTAAGCAA	494	BLAZ_(1913827..1914672)_628_659_R	TAGTCTTTTGGAACACCGTCTTTAATTAAAGT	926
2169	BLAZ_(1913827..1914672)_507_531_F	TGATACTTCAACGCCTGCTGCTTTC	467	BLAZ_(1913827..1914672)_622_651_R	TGGAACACCGTCTTTAATTAAAGTATCTCC	1263
2170	BLAZ_(1913827..1914672)_508_531_F	TATACTTCAACGCCTGCTGCTTTC	232	BLAZ_(1913827..1914672)_553_583_R	TCTTTTCTTTGCTTAATTTTCCATTTGCGAT	1145
2171	BLAZ_(1913827..1914672)_24_56_F	TGCAATTGCTTTAGTTTTAAGTGCATGTAATTC	487	BLAZ_(1913827..1914672)_121_154_R	TTACTTCCTTACCACTTTTAGTATCTAAAGCATA	1366
2172	BLAZ_(1913827..1914672)_26_58_F	TCCTTGCTTTAGTTTTAAGTGCATGTAATTCAA	351	BLAZ_(1913827..1914672)_127_157_R	TGGGGACTTCCTTACCACTTTTAGTATCTAA	1289
2173	BLAZ_NC002952-1913827-1914672_546_575_F	TCCACTTATCGCAAATGGAAAATTAAGCAA	312	BLAZ_NC002952-1913827-1914672_655_683_R	TGGCCACTTTTATCAGCAACCTTACAGTC	1277
2174	BLAZ_NC002952-1913827-1914672_546_575_2_F	TGCACTTATCGCAAATGGAAAATTAAGCAA	494	BLAZ_NC002952-1913827-1914672_628_659_R	TAGTCTTTTGGAACACCGTCTTTAATTAAAGT	926
2175	BLAZ_NC002952-1913827-1914672_507_531_F	TGATACTTCAACGCCTGCTGCTTTC	467	BLAZ_NC002952-1913827-1914672_622_651_R	TGGAACACCGTCTTTAATTAAAGTATCTCC	1263

2176	BLAZ_NC002952-1913827-1914672_508_531_F	TATACTTCAACGCCTGCTGCTTTC	232	BLAZ_NC002952-1913827-1914672_553_583_R	TCTTTTCTTTGCTTAATTTTCCATTTGCGAT	1145
2177	BLAZ_NC002952-1913827-1914672_24_56_F	TGCAATTGCTTTAGTTTTAAGTGCATGTAATTC	487	BLAZ_NC002952-1913827-1914672_121_154_R	TTACTTCCTTACCACTTTTAGTATCTAAAGCATA	1366
2178	BLAZ_NC002952-1913827-1914672_26_58_F	TCCTTGCTTTAGTTTTAAGTGCATGTAATTCAA	351	BLAZ_NC002952-1913827-1914672_127_157_R	TGGGGACTTCCTTACCACTTTTAGTATCTAA	1289
2247	TUFB_NC002758-615038-616222_693_721_F	TGTTGAACGTGGTCAAATCAAAGTTGGTG	643	TUFB_NC002758-615038-616222_793_820_R	TGTCACCAGCTTCAGCGTAGTCTAATAA	1321
2248	TUFB_NC002758-615038-616222_690_716_F	TCGTGTTGAACGTGGTCAAATCAAAGT	386	TUFB_NC002758-615038-616222_793_820_R	TGTCACCAGCTTCAGCGTAGTCTAATAA	1321
2249	TUFB_NC002758-615038-616222_696_725_F	TGAACGTGGTCAAATCAAAGTTGGTGAAGA	430	TUFB_NC002758-615038-616222_793_820_R	TGTCACCAGCTTCAGCGTAGTCTAATAA	1321
2250	TUFB_NC002758-615038-616222_488_513_F	TCCCAGGTGACGATGTACCTGTAATC	320	TUFB_NC002758-615038-616222_601_630_R	TGGTTTGTCAGAATCACGTTCTGGAGTTGG	1311
2251	TUFB_NC002758-615038-616222_945_972_F	TGAAGGTGGACGTCACACTCCATTCTTC	433	TUFB_NC002758-615038-616222_1030_1060_R	TAGGCATAACCATTTCAGTACCTTCTGGTAA	922
2252	TUFB_NC002758-615038-616222_333_356_F	TCCAATGCCACAAACTCGTGAACA	307	TUFB_NC002758-615038-616222_424_459_R	TTCCATTTCAACTAATTCTAATAATTCTTCATCGTC	1382
2253	NUC_NC002758-894288-894974_402_424_F	TCCTGAAGCAAGTGCATTTACGA	342	NUC_NC002758-894288-894974_483_509_R	TACGCTAAGCCACGTCCATATTTATCA	899
2254	NUC_NC002758-894288-894974_53_81_F	TCCTTATAGGGATGGCTATCAGTAATGTT	349	NUC_NC002758-894288-894974_165_189_R	TGTTTGTGATGCATTTGCTGAGCTA	1354
2255	NUC_NC002758-894288-894974_169_194_F	TCAGCAAATGCATCACAAACAGATAA	273	NUC_NC002758-894288-894974_222_250_R	TAGTTGAAGTTGCACTATATACTGTTGGA	928
2256	NUC_NC002758-894288-894974_316_345_F	TACAAAGGTCAACCAATGACATTCAGACTA	174	NUC_NC002758-894288-894974_396_421_R	TAAATGCACTTGCTTCAGGGCCATAT	853
2270	RPOB_EC_3798_3821_1_F	TGGCCAGCGCTTCGGTGAAATGGA	566	RPOB_EC_3868_3895_R	TCACGTCGTCCGACTTCACGGTCAGCAT	979
2271	RPOB_EC_3789_3812_F	TCAGTTCGGCGGTCAGCGCTTCGG	294	RPOB_EC_3860_3890_R	TCGTCGGACTTAACGGTCAGCATTTCCTGCA	1107
2272	RPOB_EC_3789_3812_F	TCAGTTCGGCGGTCAGCGCTTCGG	294	RPOB_EC_3860_3890_2_R	TCGTCCGACTTAACGGTCAGCATTTCCTGCA	1102

2273	RPOB_EC_3789_3812_F	TCAGTTCGGCGGTCAGCGCTTCGG	294	RPOB_EC_3862_3890_R	TCGTCGGACTTAACGGTCAGCATTTCCTG	1106
2274	RPOB_EC_3789_3812_F	TCAGTTCGGCGGTCAGCGCTTCGG	294	RPOB_EC_3862_3890_2_R	TCGTCCGACTTAACGGTCAGCATTTCCTG	1101
2275	RPOB_EC_3793_3812_F	TTCGGCGGTCAGCGCTTCGG	674	RPOB_EC_3865_3890_R	TCGTCGGACTTAACGGTCAGCATTTC	1105
2276	RPOB_EC_3793_3812_F	TTCGGCGGTCAGCGCTTCGG	674	RPOB_EC_3865_3890_2_R	TCGTCCGACTTAACGGTCAGCATTTC	1100
2309	MUPR_X75439_1658_1689_F	TCCTTTGATATATTATGCGATGGAAGGTTGGT	352	MUPR_X75439_1744_1773_R	TCCCTTCCTTAATATGAGAAGGAAACCACT	1030
2310	MUPR_X75439_1330_1353_F	TTCCTCCTTTTGAAAGCGACGGTT	669	MUPR_X75439_1413_1441_R	TGAGCTGGTGCTATATGAACAATACCAGT	1171
2312	MUPR_X75439_1314_1338_F	TTTCCTCCTTTTGAAAGCGACGGTT	704	MUPR_X75439_1381_1409_R	TATATGAACAATACCAGTTCCTTCTGAGT	931
2313	MUPR_X75439_2486_2516_F	TAATTGGGCTCTTTCTCGCTTAAACACCTTA	172	MUPR_X75439_2548_2574_R	TTAATCTGGCTGCGGAAGTGAAATCGT	1360
2314	MUPR_X75439_2547_2572_F	TACGATTTCACTTCCGCAGCCAGATT	188	MUPR_X75439_2605_2630_R	TCGTCCTCTCGAATCTCCGATATACC	1103
2315	MUPR_X75439_2666_2696_F	TGCGTACAATACGCTTTATGAAATTTTAACA	513	MUPR_X75439_2711_2740_R	TCAGATATAAATGGAACAAATGGAGCCACT	981
2316	MUPR_X75439_2813_2843_F	TAATCAAGCATTGGAAGATGAAATGCATACC	165	MUPR_X75439_2867_2890_R	TCTGCATTTTTGCGAGCCTGTCTA	1127
2317	MUPR_X75439_884_914_F	TGACATGGACTCCCCCTATATAACTCTTGAG	447	MUPR_X75439_977_1007_R	TGTACAATAAGGAGTCACCTTATGTCCCTTA	1317
2318	CTXA_NC002505-1568114-1567341_114_142_F	TGGTCTTATGCCAAGAGGACAGAGTGAGT	608	CTXA_NC002505-1568114-1567341_194_221_R	TCGTGCCTAACAAATCCCGTCTGAGTTC	1109
2319	CTXA_NC002505-1568114-1567341_117_145_F	TCTTATGCCAAGAGGACAGAGTGAGTACT	411	CTXA_NC002505-1568114-1567341_194_221_R	TCGTGCCTAACAAATCCCGTCTGAGTTC	1109
2320	CTXA_NC002505-1568114-1567341_114_142_F	TGGTCTTATGCCAAGAGGACAGAGTGAGT	608	CTXA_NC002505-1568114-1567341_186_214_R	TAACAAATCCCGTCTGAGTTCCTCTTGCA	855
2321	CTXA_NC002505-1568114-1567341_117_145_F	TCTTATGCCAAGAGGACAGAGTGAGTACT	411	CTXA_NC002505-1568114-1567341_186_214_R	TAACAAATCCCGTCTGAGTTCCTCTTGCA	855
2322	CTXA_NC002505-1568114-1567341_129_156_F	AGGACAGAGTGAGTACTTTGACCGAGGT	27	CTXA_NC002505-1568114-1567341_180_207_R	TCCCGTCTGAGTTCCTCTTGCATGATCA	1027
2323	CTXA_NC002505-1568114-1567341_122_149_F	TGCCAAGAGGACAGAGTGAGTACTTTGA	500	CTXA_NC002505-1568114-1567341_186_214_R	TAACAAATCCCGTCTGAGTTCCTCTTGCA	855
2324	INV_U22457-74-3772_831_858_F	TGCTTATTTACCTGCACTCCCACAACTG	530	INV_U22457-74-3772_942_966_R	TGACCCAAAGCTGAAAGCTTTACTG	1154
2325	INV_U22457-74-3772_827_857_F	TGAATGCTTATTTACCTGCACTCCCACAACT	438	INV_U22457-74-3772_942_970_R	TAACTGACCCAAAGCTGAAAGCTTTACTG	864
2326	INV_U22457-74-	TGCTGGTAACAGAGCCTTATAGGCGC	526	INV_U22457-74-	TGGGTTGCGTTGCAGATTATCTTTACCA	1296

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	3772_1555_1581_F	A		3772_1619_1647_R	A	
2327	INV_U22457-74-3772_1558_1585_F	TGGTAACAGAGCCTTATAGGCGCATATG	598	INV_U22457-74-3772_1622_1652_R	TCATAAGGGTTGCGTTGCAGATTATCTTTAC	987
2328	ASD_NC006570-439714-438608_3_37_F	TGAGGGTTTTATGCTTAAAGTTGGTTTTATTGGTT	459	ASD_NC006570-439714-438608_54_84_R	TGATTCGATCATACGAGACATTAAAACTGAG	1188
2329	ASD_NC006570-439714-438608_18_45_F	TAAAGTTGGTTTTATTGGTTGGCGCGGA	149	ASD_NC006570-439714-438608_66_95_R	TCAAAATCTTTTGATTCGATCATACGAGAC	948
2330	ASD_NC006570-439714-438608_17_45_F	TTAAAGTTGGTTTTATTGGTTGGCGCGGA	647	ASD_NC006570-439714-438608_67_95_R	TCCCAATCTTTTGATTCGATCATACGAGA	1016
2331	ASD_NC006570-439714-438608_9_40_F	TTTTATGCTTAAAGTTGGTTTTATTGGTTGGC	709	ASD_NC006570-439714-438608_107_134_R	TCTGCCTGAGATGTCGAAAAAAACGTTG	1128
2332	GALE_AF513299_171_200_F	TCAGCTAGACCTTTTAGGTAAAGCTAAGCT	280	GALE_AF513299_241_271_R	TCTCACCTACAGCTTTAAAGCCAGCAAAATG	1122
2333	GALE_AF513299_168_199_F	TTATCAGCTAGACCTTTTAGGTAAAGCTAAGC	658	GALE_AF513299_245_271_R	TCTCACCTACAGCTTTAAAGCCAGCAA	1121
2334	GALE_AF513299_168_199_F	TTATCAGCTAGACCTTTTAGGTAAAGCTAAGC	658	GALE_AF513299_233_264_R	TACAGCTTTAAAGCCAGCAAAATGAATTACAG	883
2335	GALE_AF513299_169_198_F	TCCCAGCTAGACCTTTTAGGTAAAGCTAAG	319	GALE_AF513299_252_279_R	TTCAACACTCTCACCTACAGCTTTAAAG	1374
2336	PLA_AF053945_7371_7403_F	TTGAGAAGACATCCGGCTCACGTTATTATGGTA	680	PLA_AF053945_7434_7468_R	TACGTATGTAAATTCCGCAAAGACTTTGGCATTAG	900
2337	PLA_AF053945_7377_7403_F	TGACATCCGGCTCACGTTATTATGGTA	443	PLA_AF053945_7428_7455_R	TCCGCAAAGACTTTGGCATTAGGTGTGA	1035
2338	PLA_AF053945_7377_7404_F	TGACATCCGGCTCACGTTATTATGGTAC	444	PLA_AF053945_7430_7460_R	TAAATTCCGCAAAGACTTTGGCATTAGGTGT	854
2339	CAF_AF053947_33412_33441_F	TCCGTTATCGCCATTGCATTATTTGGAACT	329	CAF_AF053947_33498_33523_R	TAAGAGTGATGCGGGCTGGTTCAACA	866
2340	CAF_AF053947_33426_33458_F	TGCATTATTTGGAACTATTGCAACTGCTAATGC	499	CAF_AF053947_33483_33507_R	TGGTTCAACAAGAGTTGCCGTTGCA	1308
2341	CAF_AF053947_33407_33429_F	TCAGTTCCGTTATCGCCATTGCA	291	CAF_AF053947_33483_33504_R	TTCAACAAGAGTTGCCGTTGCA	1373
2342	CAF_AF053947_33407_33431_F	TCAGTTCCGTTATCGCCATTGCATT	293	CAF_AF053947_33494_33517_R	TGATGCGGGCTGGTTCAACAAGAG	1184
2344	GAPA_NC_002505_1_28_F_1	TCAATGAACGATCAACAAGTGATTGATG	260	GAPA_NC_002505_29_58_R_1	TCCTTTATGCAACTTGGTATCAACAGGAAT	1060
2472	OMPA_NC000117_68_89_F	TGCCTGTAGGGAATCCTGCTGA	507	OMPA_NC000117_145_167_R	TCACACCAAGTAGTGCAAGGATC	967
2473	OMPA_NC000117_798_821_F	TGATTACCATGAGTGGCAAGCAAG	475	OMPA_NC000117_865_893_R	TCAAAACTTGCTCTAGACCATTTAACTCC	947
2474	OMPA_NC000117_645_671_F	TGCTCAATCTAAACCTAAAGTCGAAGA	521	OMPA_NC000117_757_777_R	TGTCGCAGCATCTGTTCCTGC	1328

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2475	OMPA_NC000117_947_973_F	TAACTGCATGGAACCCTTCTTTACTAG	157	OMPA_NC000117_1011_1040_R	TGACAGGACACAATCTGCATGAAGTCTGAG	1153
2476	OMPA_NC000117_774_795_F	TACTGGAACAAAGTCTGCGACC	196	OMPA_NC000117_871_894_R	TTCAAAAGTTGCTCGAGACCATTG	1371
2477	OMPA_NC000117_457_483_F	TTCTATCTCGTTGGTTTATTCGGAGTT	676	OMPA_NC000117_511_534_R	TAAAGAGACGTTTGGTAGTTCATTTGC	851
2478	OMPA_NC000117_687_710_F	TAGCCCAGCACAATTTGTGATTCA	212	OMPA_NC000117_787_816_R	TTGCCATTCATGGTATTTAAGTGTAGCAGA	1406
2479	OMPA_NC000117_540_566_F	TGGCGTAGTAGAGCTATTTACAGACAC	571	OMPA_NC000117_649_672_R	TTCTTGAACGCGAGGTTTCGATTG	1395
2480	OMPA_NC000117_338_360_F	TGCACGATGCGGAATGGTTCACA	492	OMPA_NC000117_417_444_R	TCCTTTAAAATAACCGCTAGTAGCTCCT	1058
2481	OMP2_NC000117_18_40_F	TATGACCAAACTCATCAGACGAG	234	OMP2_NC000117_71_91_R	TCCCGCTGGCAAATAAACTCG	1025
2482	OMP2_NC000117_354_382_F	TGCTACGGTAGGATCTCCTTATCCTATTG	516	OMP2_NC000117_445_471_R	TGGATCACTGCTTACGAACTCAGCTTC	1270
2483	OMP2_NC000117_1297_1319_F	TGGAAAGGTGTTGCAGCTACTCA	537	OMP2_NC000117_1396_1419_R	TACGTTTGTATCTTCTGCAGAACC	903
2484	OMP2_NC000117_1465_1493_F	TCTGGTCCAACAAAAGGAACGATTACAGG	407	OMP2_NC000117_1541_1569_R	TCCTTTCAATGTTACAGAAAACTCTACAG	1062
2485	OMP2_NC000117_44_66_F	TGACGATCTTCGCGGTGACTAGT	450	OMP2_NC000117_120_148_R	TGTCAGCTAAGCTAATAACGTTTGTAGAG	1323
2486	OMP2_NC000117_166_190_F	TGACAGCGAAGAAGGTTAGACTTGTCC	441	OMP2_NC000117_240_261_R	TTGACATCGTCCCTCTTCACAG	1396
2487	GYRA_NC000117_514_536_F	TCAGGCATTGCGGTTGGGATGGC	287	GYRA_NC000117_640_660_R	TGCTGTAGGGAAATCAGGGCC	1251
2488	GYRA_NC000117_801_827_F	TGTGAATAAATCACGATTGATTGAGCA	636	GYRA_NC000117_871_893_R	TTGTCAGACTCATCGCGAACATC	1419
2489	GYRA_NC002952_219_242_F	TGTCATGGGTAAATATCACCCTCA	632	GYRA_NC002952_319_345_R	TCCATCCATAGAACCAAAGTTACCTTG	1010
2490	GYRA_NC002952_964_983_F	TACAAGCACTCCCAGCTGCA	176	GYRA_NC002952_1024_1041_R	TCGCAGCGTGCGTGGCAC	1073
2491	GYRA_NC002952_1505_1520_F	TCGCCCGCGAGGACGT	366	GYRA_NC002952_1546_1562_R	TTGGTGCGCTTGGCGTA	1416
2492	GYRA_NC002952_59_81_F	TCAGCTACATCGACTATGCGATG	279	GYRA_NC002952_124_143_R	TGGCGATGCACTGGCTTGAG	1279
2493	GYRA_NC002952_216_239_F	TGACGTCATCGGTAAGTACCACCC	452	GYRA_NC002952_313_333_R	TCCGAAGTTGCCCTGGCCGTC	1032
2494	GYRA_NC002952_219_242_2_F	TGTACTCGGTAAGTATCACCCGCA	625	GYRA_NC002952_308_330_R	TAAGTTACCTTGCCCGTCAACCA	873
2495	GYRA_NC002952_115_141_F	TGAGATGGATTTAAACCTGTTCACCGC	453	GYRA_NC002952_220_242_R	TGCGGGTGATACTTACCGAGTAC	1236
2496	GYRA_NC002952_517_539_F	TCAGGCATTGCGGTTGGGATGGC	287	GYRA_NC002952_643_663_R	TGCTGTAGGGAAATCAGGGCC	1251
2497	GYRA_NC002952_273_2	TCGTATGGCTCAATGGTGGAG	380	GYRA_NC002952_338_360_R	TGCGGCAGCACTATCACCATCCA	1234

	93_F					
2498	GYRA_NC000912_257_278_F	TGAGTAAGTTCCACCCGCACGG	462	GYRA_NC000912_346_370_R	TCGAGCCGAAGTTACCCTGTCCGTC	1067
2504	ARCC_NC003923-2725050-2724595_135_161P_F	TAGTpGATpAGAACpTpGTAGGCpACpAATpCpGT	229	ARCC_NC003923-2725050-2724595_214_239P_R	TCpTpTpTpCpGTATAAAAAGGACpCpAATpTpGG	1116
2505	PTA_NC003923-628885-629355_237_263P_F	TCTTGTpTpTpATGCpTpGGTAAAGCAGATGG	417	PTA_NC003923-628885-629355_314_342P_R	TACpACpCpTGGTpTpTpCpGTpTpTpTpGATGATpTpTpGTA	904
2517	CJMLST_ST1_1852_1883_F	TTTGCGGATGAAGTAGGTGCCTATCTTTTTGC	708	CJMLST_ST1_1945_1977_R	TGTTTTATGTGTAGTTGAGCTTACTACATGAGC	1355
2518	CJMLST_ST1_2963_2992_F	TGAAATTGCTACAGGCCCTTTAGGACAAGG	428	CJMLST_ST1_3073_3097_R	TCCCCATCTCCGCAAAGACAATAAA	1020
2519	CJMLST_ST1_2350_2378_F	TGCTTTTGATGGTGATGCAGATCGTTTGG	535	CJMLST_ST1_2447_2481_R	TCTACAACACTTGATTGTAATTTGCCTTGTTCTTT	1117
2520	CJMLST_ST1_654_684_F	TATGTCCAAGAAGCATAGCAAAAAAAGCAAT	240	CJMLST_ST1_725_756_R	TCGGAAACAAAGAATTCATTTTCTGGTCCAAA	1084
2521	CJMLST_ST1_360_395_F	TCCTGTTATTCCTGAAGTAGTTAATCAAGTTTGTTA	347	CJMLST_ST1_454_487_R	TGCTATATGCTACAACTGGTTCAAAAACATTAAG	1245
2522	CJMLST_ST1_1231_1258_F	TGGCAGTTTTACAAGGTGCTGTTTCATC	564	CJMLST_ST1_1312_1340_R	TTTAGCTACTATTCTAGCTGCCATTTCCA	1427
2523	CJMLST_ST1_3543_3574_F	TGCTGTAGCTTATCGCGAAATGTCTTTGATTT	529	CJMLST_ST1_3656_3685_R	TCAAAGAACCAGCACCTAATTCATCATTTA	950
2524	CJMLST_ST1_1_17_F	TAAAACTTTTGCCGTAATGATGGGTGAAGATAT	145	CJMLST_ST1_55_84_R	TGTTCCAATAGCAGTTCCGCCCAAATTGAT	1348
2525	CJMLST_ST1_1312_1342_F	TGGAAATGGCAGCTAGAATAGTAGCTAAAAT	538	CJMLST_ST1_1383_1417_R	TTTCCCCGATCTAAATTTGGATAAGCCATAGGAAA	1432
2526	CJMLST_ST1_2254_2286_F	TGGGCCTAATGGGCTTAATATCAATGAAAATTG	582	CJMLST_ST1_2352_2379_R	TCCAAACGATCTGCATCACCATCAAAAG	996
2527	CJMLST_ST1_1380_1411_F	TGCTTTCCTATGGCTTATCCAAATTTAGATCG	534	CJMLST_ST1_1486_1520_R	TGCATGAAGCATAAAAACTGTATCAAGTGCTTTTA	1205
2528	CJMLST_ST1_3413_3437_F	TTGTAAATGCCGGTGCTTCAGATCC	692	CJMLST_ST1_3511_3542_R	TGCTTGCTCAAATCATCATAAACAATTAAAGC	1257
2529	CJMLST_ST1_1130_1156_F	TACGCGTCTTGAAGCGTTTCGTTATGA	189	CJMLST_ST1_1203_1230_R	TAGGATGAGCATTATCAGGGAAAGAATC	920
2530	CJMLST_ST1_2840_2872_F	TGGGGCTTTGCTTTATAGTTTTTTACATTTAAG	591	CJMLST_ST1_2940_2973_R	TAGCGATTTCTACTCCTAGAGTTGAAATTTCAGG	917
2531	CJMLST_ST1_2058_2084_F	TATTCAAGGTGGTCCTTTGATGCATGT	241	CJMLST_ST1_2131_2162_R	TTGGTTCTTACTTGTTTTGCATAAACTTTCCA	1417
2532	CJMLST_ST1_553_585_F	TCCTGATGCTCAAAGTGCTTTTTTAGATCCTTT	344	CJMLST_ST1_655_685_R	TATTGCTTTTTTTGCTATGCTTCTTGGACAT	942
2564	GLTA_NC002163-1604930-1604529_306_338_F	TCATGTTGAGCTTAAACCTATAGAAGTAAAAGC	299	GLTA_NC002163-1604930-1604529_352_380_R	TTTTGCTCATGATCTGCATGAAGCATAAA	1443
2565	UNCA_NC002163-	TCCCCCACGCTTTAATTGTTTATGAT	322	UNCA_NC002163-112166-	TCGACCTGGAGGACGACGTAAAATCA	1065

	112166-112647_80_113_F	GATTTGAG		112647_146_171_R		
2566	UNCA_NC002163-112166-112647_233_259_F	TAATGATGAATTAGGTGCGGGTTCTTT	170	UNCA_NC002163-112166-112647_294_329_R	TGGGATAACATTGGTTGGAATATAAGCAGAAACATC	1285
2567	PGM_NC002163-327773-328270_273_305_F	TCTTGATACTTGTAATGTGGGCGATAAATATGT	414	PGM_NC002163-327773-328270_365_396_R	TCCATCGCCAGTTTTTGCATAATCGCTAAAAA	1012
2568	TKT_NC002163-1569415-1569873_255_284_F	TTATGAAGCGTGTTCTTTAGCAGGACTTCA	661	TKT_NC002163-1569415-1569873_350_383_R	TCAAAACGCATTTTTACATCTTCGTTAAAGGCTA	946
2570	GLTA_NC002163-1604930-1604529_39_68_F	TCGTCTTTTTGATTCTTTCCCTGATAATGC	381	GLTA_NC002163-1604930-1604529_109_142_R	TGTTCATGTTTAAATGATCAGGATAAAAAGCACT	1347
2571	TKT_NC002163-1569415-1569903_33_62_F	TGATCTTAAAAATTTCCGCCAACTTCATTC	472	TKT_NC002163-1569415-1569903_139_162_R	TGCCATAGCAAAGCCTACAGCATT	1214
2572	TKT_NC002163-1569415-1569903_207_239_F	TAAGGTTTATTGTCTTTGTGGAGATGGGGATTT	164	TKT_NC002163-1569415-1569903_313_345_R	TACATCTCCTTCGATAGAAATTTCATTGCTATC	886
2573	TKT_NC002163-1569415-1569903_350_383_F	TAGCCTTTAACGAAAATGTAAAAATGCGTTTTGA	213	TKT_NC002163-1569415-1569903_449_481_R	TAAGACAAGGTTTTGTGGATTTTTTAGCTTGTT	865
2574	TKT_NC002163-1569415-1569903_60_92_F	TTCAAAAACTCCAGGCCATCCTGAAATTTCAAC	665	TKT_NC002163-1569415-1569903_139_163_R	TTGCCATAGCAAAGCCTACAGCATT	1405
2575	GLTA_NC002163-1604930-1604529_39_70_F	TCGTCTTTTTGATTCTTTCCCTGATAATGCTC	382	GLTA_NC002163-1604930-1604529_139_168_R	TGCCATTTCCATGTACTCTTCTCTAACATT	1216
2576	GLYA_NC002163-367572-368079_386_414_F	TCAGCTATTTTTCCAGGTATCCAAGGTGG	281	GLYA_NC002163-367572-368079_476_508_R	ATTGCTTCTTACTTGCTTAGCATAAATTTTCCA	756
2577	GLYA_NC002163-367572-368079_148_174_F	TGGTGCGAGTGCTTATGCTCGTATTAT	611	GLYA_NC002163-367572-368079_242_270_R	TGCTCACCTGCTACAACAAGTCCAGCAAT	1246
2578	GLYA_NC002163-367572-368079_298_327_F	TGTAAGCTCTACAACCCACAAAACCTTACG	622	GLYA_NC002163-367572-368079_384_416_R	TTCCACCTTGGATACCTGGAAAAATAGCTGAAT	1381
2579	GLYA_NC002163-367572-368079_1_27_F	TGGTGGACATTTAACACATGGTGCAAA	614	GLYA_NC002163-367572-368079_52_81_R	TCAAGCTCTACACCATAAAAAAAGCTCTCA	961
2580	PGM_NC002163-327746-328270_254_285_F	TGAGCAATGGGGCTTTGAAAGAATTTTTAAAT	455	PGM_NC002163-327746-328270_356_379_R	TTTGCTCTCCGCCAAAGTTTCCAC	1438
2581	PGM_NC002163-327746-	TGAAAAGGGTGAAGTAGCAAATGGAGATAG	425	PGM_NC002163-327746-328270_241_267_R	TGCCCCATTGCTCATGATAGTAGCTAC	1219

	328270_153_182_F					
2582	PGM_NC002163-327746-328270_19_50_F	TGGCCTAATGGGCTTAATATCAATGAAAATTG	568	PGM_NC002163-327746-328270_79_102_R	TGCACGCAAACGCTTTACTTCAGC	1200
2583	UNCA_NC002163-112166-112647_114_141_F	TAAGCATGCTGTGGCTTATCGTGAAATG	160	UNCA_NC002163-112166-112647_196_225_R	TGCCCTTTCTAAAAGTCTTGAGTGAAGATA	1220
2584	UNCA_NC002163-112166-112647_3_29_F	TGCTTCGGATCCAGCAGCACTTCAATA	532	UNCA_NC002163-112166-112647_88_123_R	TGCATGCTTACTCAAATCATCATAAACAATTAAAGC	1206
2585	ASPA_NC002163-96692-97166_308_335_F	TTAATTTGCCAAAAATGCAACCAGGTAG	652	ASPA_NC002163-96692-97166_403_432_R	TGCAAAAGTAACGGTTACATCTGCTCCAAT	1192
2586	ASPA_NC002163-96692-97166_228_258_F	TCGCGTTGCAACAAAACTTTCTAAAGTATGT	370	ASPA_NC002163-96692-97166_316_346_R	TCATGATAGAACTACCTGGTTGCATTTTTGG	991
2587	GLNA_NC002163-658085-657609_244_275_F	TGGAATGATGATAAAGATTTCGCAGATAGCTA	547	GLNA_NC002163-658085-657609_340_371_R	TGAGTTTGAACCATTTCAGAGCGAATATCTAC	1176
2588	TKT_NC002163-1569415-1569903_107_130_F	TCGCTACAGGCCCTTTAGGACAAG	371	TKT_NC002163-1569415-1569903_212_236_R	TCCCCATCTCCGCAAAGACAATAAA	1020
2589	TKT_NC002163-1569415-1569903_265_296_F	TGTTCTTTAGCAGGACTTCACAAACTTGATAA	642	TKT_NC002163-1569415-1569903_361_393_R	TCCTTGTGCTTCAAAACGCATTTTTACATTTTC	1057
2590	GLYA_NC002163-367572-368095_214_246_F	TGCCTATCTTTTTGCTGATATAGCACATATTGC	505	GLYA_NC002163-367572-368095_317_340_R	TCCTCTTGGGCCACGCAAAGTTTT	1047
2591	GLYA_NC002163-367572-368095_415_444_F	TCCTTTGATGCATGTAATTGCTGCAAAAGC	353	GLYA_NC002163-367572-368095_485_516_R	TCTTGAGCATTGGTTCTTACTTGTTTTGCATA	1141
2592	PGM_NC002163_21_54_F	TCCTAATGGACTTAATATCAATGAAAATTGTGGA	332	PGM_NC002163_116_142_R	TCAAACGATCCGCATCACCATCAAAAG	949
2593	PGM_NC002163_149_176_F	TAGATGAAAAAGGCGAAGTGGCTAATGG	207	PGM_NC002163_247_277_R	TCCCCTTTAAAGCACCATTACTCATTATAGT	1023
2594	GLNA_NC002163-658085-657609_79_106_F	TGTCCAAGAAGCATAGCAAAAAAAGCAA	633	GLNA_NC002163-658085-657609_148_179_R	TCAAAAACAAAGAATTCATTTTCTGGTCCAAA	945
2595	ASPA_NC002163-96685-97196_367_402_F	TCCTGTTATTCCTGAAGTAGTTAATCAAGTTTGTTA	347	ASPA_NC002163-96685-97196_467_497_R	TCAAGCTATATGCTACAACTGGTTCAAAAAC	960
2596	ASPA_NC002163-96685-97196_1_33_F	TGCCGTAATGATAGGTGAAGATATACAAAGAGT	502	ASPA_NC002163-96685-97196_95_127_R	TACAACCTTCGGATAATCAGGATGAGAATTAAT	880
2597	ASPA_NC002163-96685-	TGGAACAGGAATTAATTCTCATCCTGATTATCC	540	ASPA_NC002163-96685-97196_185_210_R	TAAGCTCCCGTATCTTGAGTCGCCTC	872

	97196_85_117_F					
2598	PGM_NC002163-327746-328270_165_195_F	TGGCAGCTAGAATAGTAGCTAAAATCCCTAC	563	PGM_NC002163-327746-328270_230_261_R	TCACGATCTAAATTTGGATAAGCCATAGGAAA	975
2599	PGM_NC002163-327746-328270_252_286_F	TGGGTCGTGGTTTTACAGAAAATTTCTTATATATG	593	PGM_NC002163-327746-328270_353_381_R	TTTTGCTCATGATCTGCATGAAGCATAAA	1443
2600	PGM_NC002163-327746-328270_1_30_F	TGGGATGAAAAAGCGTTCTTTTATCCATGA	577	PGM_NC002163-327746-328270_95_123_R	TGATAAAAAGCACTAAGCGATGAAACAGC	1178
2601	PGM_NC002163-327746-328270_220_250_F	TAAACACGGCTTTCCTATGGCTTATCCAAAT	146	PGM_NC002163-327746-328270_314_345_R	TCAAGTGCTTTTACTTCTATAGGTTTAAGCTC	963
2602	UNCA_NC002163-112166-112647_123_152_F	TGTAGCTTATCGCGAAATGTCTTTGATTTT	628	UNCA_NC002163-112166-112647_199_229_R	TGCTTGCTCTTTCAAGCAGTCTTGAATGAAG	1258
2603	UNCA_NC002163-112166-112647_333_365_F	TCCAGATGGACAAATTTTCTTAGAAACTGATTT	313	UNCA_NC002163-112166-112647_430_461_R	TCCGAAACTTGTTTTGTAGCTTTAATTTGAGC	1031
2734	GYRA_AY291534_237_264_F	TCACCCTCATGGTGATTCAGCTGTTTAT	265	GYRA_AY291534_268_288_R	TTGCGCCATACGTACCATCGT	1407
2735	GYRA_AY291534_224_252_F	TAATCGGTAAGTATCACCCTCATGGTGAT	167	GYRA_AY291534_256_285_R	TGCCATACGTACCATCGTTTCATAAACAGC	1213
2736	GYRA_AY291534_170_198_F	TAGGAATTACGGCTGATAAAGCGTATAAA	221	GYRA_AY291534_268_288_R	TTGCGCCATACGTACCATCGT	1407
2737	GYRA_AY291534_224_252_F	TAATCGGTAAGTATCACCCTCATGGTGAT	167	GYRA_AY291534_319_346_R	TATCGACAGATCCAAAGTTACCATGCCC	935
2738	GYRA_NC002953-7005-9668_166_195_F	TAAGGTATGACACCGGATAAATCATATAAA	163	GYRA_NC002953-7005-9668_265_287_R	TCTTGAGCCATACGTACCATTGC	1142
2739	GYRA_NC002953-7005-9668_221_249_F	TAATGGGTAAATATCACCCTCATGGTGAC	171	GYRA_NC002953-7005-9668_316_343_R	TATCCATTGAACCAAAGTTACCTTGGCC	933
2740	GYRA_NC002953-7005-9668_221_249_F	TAATGGGTAAATATCACCCTCATGGTGAC	171	GYRA_NC002953-7005-9668_253_283_R	TAGCCATACGTACCATTGCTTCATAAATAGA	912
2741	GYRA_NC002953-7005-9668_234_261_F	TCACCCTCATGGTGACTCATCTATTTAT	264	GYRA_NC002953-7005-9668_265_287_R	TCTTGAGCCATACGTACCATTGC	1142
2842	CAPC_AF188935-56074-55628_271_304_F	TGGGATTATTGTTATCCTGTTATGCCATTTGAGA	578	CAPC_AF188935-56074-55628_348_378_R	TGGTAACCCTTGTCTTTGAATTGTATTTGCA	1299
2843	CAPC_AF188935-56074-55628_273_303P_F	TGATTATTGTTATCCTGTTATGCpCpATpTpTpGAG	476	CAPC_AF188935-56074-55628_349_377P_R	TGTAACCCTTGTCTTTGAATpTpGTATpTpTpGC	1314
2844	CAPC_AF188935-56074-55628_268_303_F	TCCGTTGATTATTGTTATCCTGTTATGCCATTTGAG	331	CAPC_AF188935-56074-55628_349_384_R	TGTTAATGGTAACCCTTGTCTTTGAATTGTATTTGC	1344
2845	CAPC_AF188935-	TCCGTTGATTATTGTTATCCTGTTAT	331	CAPC_AF188935-56074-	TAACCCTTGTCTTTGAATTGTATTTGCA	860

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	56074-55628_268_303_F	GCCATTTGAG		55628_337_375_R	ATTAATCCTGG	
2846	PARC_X95819_33_58_F	TCCAAAAAAATCAGCGCGTACAGTGG	302	PARC_X95819_121_153_R	TAAAGGATAGCGGTAACTAAATGGCTGAGCCAT	852
2847	PARC_X95819_65_92_F	TACTTGGTAAATACCACCCACATGGTGA	199	PARC_X95819_157_178_R	TACCCCAGTTCCCCTGACCTTC	889
2848	PARC_X95819_69_93_F	TGGTAAATACCACCCACATGGTGAC	596	PARC_X95819_97_128_R	TGAGCCATGAGTACCATGGCTTCATAACATGC	1169
2849	PARC_NC003997-3362578-3365001_181_205_F	TTCCGTAAGTCGGCTAAAACAGTCG	668	PARC_NC003997-3362578-3365001_256_283_R	TCCAAGTTTGACTTAAACGTACCATCGC	1001
2850	PARC_NC003997-3362578-3365001_217_240_F	TGTAACTATCACCCGCACGGTGAT	621	PARC_NC003997-3362578-3365001_304_335_R	TCGTCAACACTACCATTATTACCATGCATCTC	1099
2851	PARC_NC003997-3362578-3365001_217_240_F	TGTAACTATCACCCGCACGGTGAT	621	PARC_NC003997-3362578-3365001_244_275_R	TGACTTAAACGTACCATCGCTTCATATACAGA	1162
2852	GYRA_AY642140_-1_24_F	TAAATCTGCCCGTGTCGTTGGTGAC	150	GYRA_AY642140_71_100_R	TGCTAAAGTCTTGAGCCATACGAACAATGG	1242
2853	GYRA_AY642140_26_54_F	TAATCGGTAAATATCACCCGCATGGTGAC	166	GYRA_AY642140_121_146_R	TCGATCGAACCGAAGTTACCCTGACC	1069
2854	GYRA_AY642140_26_54_F	TAATCGGTAAATATCACCCGCATGGTGAC	166	GYRA_AY642140_58_89_R	TGAGCCATACGAACAATGGTTTCATAAACAGC	1168
2860	CYA_AF065404_1348_1379_F	TCCAACGAAGTACAATACAAGACAAAAGAAGG	305	CYA_AF065404_1448_1472_R	TCAGCTGTTAACGGCTTCAAGACCC	983
2861	LEF_BA_AF065404_751_781_F	TCGAAAGCTTTTGCATATTATATCGAGCCAC	354	LEF_BA_AF065404_843_881_R	TCTTTAAGTTCTTCCAAGGATAGATTTATTTCTTGTTCG	1144
2862	LEF_BA_AF065404_762_788_F	TGCATATTATATCGAGCCACAGCATCG	498	LEF_BA_AF065404_843_881_R	TCTTTAAGTTCTTCCAAGGATAGATTTATTTCTTGTTCG	1144
2917	MUTS_AY698802_106_125_F	TCCGCTGAATCTGTCGCCGC	326	MUTS_AY698802_172_193_R	TGCGGTCTGGCGCATATAGGTA	1237
2918	MUTS_AY698802_172_192_F	TACCTATATGCGCCAGACCGC	187	MUTS_AY698802_228_252_R	TCAATCTCGACTTTTTGTGCCGGTA	965
2919	MUTS_AY698802_228_252_F	TACCGGCGCAAAAAGTCGAGATTGG	186	MUTS_AY698802_314_342_R	TCGGTTTCAGTCATCTCCACCATAAAGGT	1097
2920	MUTS_AY698802_315_342_F	TCTTTATGGTGGAGATGACTGAAACCGA	419	MUTS_AY698802_413_433_R	TGCCAGCGACAGACCATCGTA	1210
2921	MUTS_AY698802_394_411_F	TGGGCGTGGAACGTCCAC	585	MUTS_AY698802_497_519_R	TCCGGTAACTGGGTCAGCTCGAA	1040
2922	AB_MLST-11-OIF007_991_1018_F	TGGGcGATGCTGCgAAATGGTTAAAAGA	583	AB_MLST-11-OIF007_1110_1137_R	TAGTATCACCACGTACACCCGGATCAGT	923
2927	GAPA_NC002505_694_721_F	TCAATGAACGACCAACAAGTGATTGATG	259	GAPA_NC_002505_29_58_R_1	TCCTTTATGCAACTTGGTATCAACAGGAAT	1060
2928	GAPA_NC002505_694_721_2_F	TCGATGAACGACCAACAAGTGATTGATG	361	GAPA_NC002505_769_798_2_R	TCCTTTATGCAACTTGGTATCAACCGGAAT	1061

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2929	GAPA_NC002505_694_721_2_F	TCGATGAACGACCAACAAGTGATTGATG	361	GAPA_NC002505_769_798_3_R	TCCTTTATGCAACTTAGTATCAACCGGAAT	1059
2932	INFB_EC_1364_1394_F	TTGCTCGTGGTGCACAAGTAACGGATATTAC	688	INFB_EC_1439_1468_R	TTGCTGCTTTCGCATGGTTAATCGCTTCAA	1410
2933	INFB_EC_1364_1394_2_F	TTGCTCGTGGTGCAIAAGTAACGGATATIAC	689	INFB_EC_1439_1468_R	TTGCTGCTTTCGCATGGTTAATCGCTTCAA	1410
2934	INFB_EC_80_110_F	TTGCCCGCGGTGCGGAAGTAACCGATATTAC	685	INFB_EC_1439_1468_R	TTGCTGCTTTCGCATGGTTAATCGCTTCAA	1410
2949	ACS_NC002516-970624-971013_299_316_F	TCGGCGCCTGCCTGATGA	376	ACS_NC002516-970624-971013_364_383_R	TGGACCACGCCGAAGAACGG	1265
2950	ARO_NC002516-26883-27380_4_26_F	TCACCGTGCCGTTCAAGGAAGAG	267	ARO_NC002516-26883-27380_111_128_R	TGTGTTGTCGCCGCGCAG	1341
2951	ARO_NC002516-26883-27380_356_377_F	TTTCGAAGGGCCTTTCGACCTG	705	ARO_NC002516-26883-27380_459_484_R	TCCTTGGCATACATCATGTCGTAGCA	1056
2952	GUA_NC002516-4226546-4226174_23_41_F	TGGACTCCTCGGTGGTCGC	551	GUA_NC002516-4226546-4226174_127_146_R	TCGGCGAACATGGCCATCAC	1091
2953	GUA_NC002516-4226546-4226174_120_142_F	TGACCAGGTGATGGCCATGTTCG	448	GUA_NC002516-4226546-4226174_214_233_R	TGCTTCTCTTCCGGGTCGGC	1256
2954	GUA_NC002516-4226546-4226174_155_178_F	TTTTGAAGGTGATCCGTGCCAACG	710	GUA_NC002516-4226546-4226174_265_287_R	TGCTTGGTGGCTTCTTCGTCGAA	1259
2955	GUA_NC002516-4226546-4226174_190_206_F	TTCCTCGGCCGCCTGGC	670	GUA_NC002516-4226546-4226174_288_309_R	TGCGAGGAACTTCACGTCCTGC	1229
2956	GUA_NC002516-4226546-4226174_242_263_F	TCGGCCGCACCTTCATCGAAGT	374	GUA_NC002516-4226546-4226174_355_371_R	TCGTGGGCCTTGCCGGT	1111
2957	MUT_NC002516-5551158-5550717_5_26_F	TGGAAGTCATCAAGCGCCTGGC	545	MUT_NC002516-5551158-5550717_99_116_R	TCACGGGCCAGCTCGTCT	978
2958	MUT_NC002516-5551158-5550717_152_168_F	TCGAGCAGGCGCTGCCG	358	MUT_NC002516-5551158-5550717_256_277_R	TCACCATGCGCCCGTTCACATA	971
2959	NUO_NC002516-2984589-2984954_8_26_F	TCAACCTCGGCCCGAACCA	249	NUO_NC002516-2984589-2984954_97_117_R	TCGGTGGTGGTAGCCGATCTC	1095
2960	NUO_NC002516-2984589-2984954_218_239_F	TACTCTCGGTGGAGAAGCTCGC	195	NUO_NC002516-2984589-2984954_301_326_R	TTCAGGTACAGCAGGTGGTTCAGGAT	1376
2961	PPS_NC002516-1915014-1915383_44_63_F	TCCACGGTCATGGAGCGCTA	311	PPS_NC002516-1915014-1915383_140_165_R	TCCATTTCCGACACGTCGTTGATCAC	1014

2962	PPS_NC002516-1915014-1915383_240_258_F	TCGCCATCGTCACCAACCG	365	PPS_NC002516-1915014-1915383_341_360_R	TCCTGGCCATCCTGCAGGAT	1052
2963	TRP_NC002516-671831-672273_24_42_F	TGCTGGTACGGGTCGAGGA	527	TRP_NC002516-671831-672273_131_150_R	TCGATCTCCTTGGCGTCCGA	1071
2964	TRP_NC002516-671831-672273_261_282_F	TGCACATCGTGTCCAACGTCAC	490	TRP_NC002516-671831-672273_362_383_R	TGATCTCCATGGCGCGGATCTT	1182
2972	AB_MLST-11-OIF007_1007_1034_F	TGGGIGATGCTGCIAAATGGTTAAAAGA	592	AB_MLST-11-OIF007_1126_1153_R	TAGTATCACCACGTACICCIGGATCAGT	924
2993	OMPU_NC002505-674828-675880_428_455_F	TTCCCACCGATATCATGGCTTACCACGG	667	OMPU_NC002505_544_567_R	TCGGTCAGCAAAACGGTAGCTTGC	1094
2994	GAPA_NC002505-506780-507937_691_721_F	TCCTCAATGAACGAICAACAAGTGATTGATG	335	GAPA_NC002505-506780-507937_769_802_R	TTTTCCCTTTATGCAACTTAGTATCAACIGGAAT	1442
2995	GAPA_NC002505-506780-507937_691_721_2_F	TCCTCIATGAACGAICAACAAGTGATTGATG	339	GAPA_NC002505-506780-507937_769_803_R	TCCATACCTTTATGCAACTTIGTATCAACIGGAAT	1008
2996	GAPA_NC002505-506780-507937_692_721_F	TCTCGATGAACGACCAACAAGTGATTGATG	396	GAPA_NC002505-506780-507937_785_817_R	TCGGAAATATTCTTTCAATACCTTTATGCAACT	1085
2997	GAPA_NC002505-506780-507937_691_721_3_F	TCCTCGATGAACGAICAACAAGTIATTGATG	337	GAPA_NC002505-506780-507937_785_817_R	TCGGAAATATTCTTTCAATACCTTTATGCAACT	1085
2998	GAPA_NC002505-506780-507937_691_721_4_F	TCCTCAATGAATGATCAACAAGTGATTGATG	336	GAPA_NC002505-506780-507937_784_817_R	TCGGAAATATTCTTTCAATICCTTTITGCAACTT	1087
2999	GAPA_NC002505-506780-507937_691_721_5_F	TCCTCIATGAAIGAICAACAAGTIATTGATG	340	GAPA_NC002505-506780-507937_784_817_2_R	TCGGAAATATTCTTTCAATACCTTTATGCAACTT	1086
3000	GAPA_NC002505-506780-507937_691_721_6_F	TCCTCGATGAATGAICAACAAGTIATTGATG	338	GAPA_NC002505-506780-507937_769_805_R	TTTCAATACCTTTATGCAACTTIGTATCAACIGGAAT	1430
3001	CTXB_NC002505-1566967-1567341_46_71_F	TCAGCATATGCACATGGAACACCTCA	275	CTXB_NC002505-1566967-1567341_139_163_R	TCCCGGCTAGAGATTCTGTATACGA	1026
3002	CTXB_NC002505-1566967-1567341_46_70_F	TCAGCATATGCACATGGAACACCTC	274	CTXB_NC002505-1566967-1567341_132_162_R	TCCGGCTAGAGATTCTGTATACGAAAATATC	1038
3003	CTXB_NC002505-1566967-1567341_46_70_F	TCAGCATATGCACATGGAACACCTC	274	CTXB_NC002505-1566967-1567341_118_150_R	TGCCGTATACGAAAATATCTTATCATTTAGCGT	1225
3004	TUFB_NC002758-615038-	TACAGGCCGTGTTGAACGTGG	180	TUFB_NC002758-615038-616222_778_809_R	TCAGCGTAGTCTAATAATTTACGGAACATTTC	982

	616222_684_704_F					
3005	TUFB_NC002758-615038-616222_688_710_F	TGCCGTGTTGAACGTGGTCAAAT	503	TUFB_NC002758-615038-616222_783_813_R	TGCTTCAGCGTAGTCTAATAATTTACGGAAC	1255
3006	TUFB_NC002758-615038-616222_700_726_F	TGTGGTCAAATCAAAGTTGGTGAAGAA	638	TUFB_NC002758-615038-616222_778_807_R	TGCGTAGTCTAATAATTTACGGAACATTTC	1238
3007	TUFB_NC002758-615038-616222_702_726_F	TGGTCAAATCAAAGTTGGTGAAGAA	607	TUFB_NC002758-615038-616222_778_807_R	TGCGTAGTCTAATAATTTACGGAACATTTC	1238
3008	TUFB_NC002758-615038-616222_696_726_F	TGAACGTGGTCAAATCAAAGTTGGTGAAGAA	431	TUFB_NC002758-615038-616222_785_818_R	TCACCAGCTTCAGCGTAGTCTAATAATTTACGGA	970
3009	TUFB_NC002758-615038-616222_690_716_F	TCGTGTTGAACGTGGTCAAATCAAAGT	386	TUFB_NC002758-615038-616222_778_812_R	TCTTCAGCGTAGTCTAATAATTTACGGAACATTTC	1134
3010	MECI-R_NC003923-41798-41609_36_59_F	TCACATATCGTGAGCAATGAACTG	261	MECI-R_NC003923-41798-41609_89_112_R	TGTGATATGGAGGTGTAGAAGGTG	1332
3011	MECI-R_NC003923-41798-41609_40_66_F	TGGGCGTGAGCAATGAACTGATTATAC	584	MECI-R_NC003923-41798-41609_81_110_R	TGGGATGGAGGTGTAGAAGGTGTTATCATC	1287
3012	MECI-R_NC003923-41798-41609_33_60_2_F	TGGACACATATCGTGAGCAATGAACTGA	549	MECI-R_NC003923-41798-41609_81_110_R	TGGGATGGAGGTGTAGAAGGTGTTATCATC	1286
3013	MECI-R_NC003923-41798-41609_29_60_F	TGGGTTTACACATATCGTGAGCAATGAACTGA	595	MECI-R_NC003923-41798-41609_81_113_R	TGGGGATATGGAGGTGTAGAAGGTGTTATCATC	1290
3014	MUPR_X75439_2490_2514_F	TGGGCTCTTTCTCGCTTAAACACCT	587	MUPR_X75439_2548_2570_R	TCTGGCTGCGGAAGTGAAATCGT	1130
3015	MUPR_X75439_2490_2513_F	TGGGCTCTTTCTCGCTTAAACACC	586	MUPR_X75439_2547_2568_R	TGGCTGCGGAAGTGAAATCGTA	1281
3016	MUPR_X75439_2482_2510_F	TAGATAATTGGGCTCTTTCTCGCTTAAAC	205	MUPR_X75439_2551_2573_R	TAATCTGGCTGCGGAAGTGAAAT	876
3017	MUPR_X75439_2490_2514_F	TGGGCTCTTTCTCGCTTAAACACCT	587	MUPR_X75439_2549_2573_R	TAATCTGGCTGCGGAAGTGAAATCG	877
3018	MUPR_X75439_2482_2510_F	TAGATAATTGGGCTCTTTCTCGCTTAAAC	205	MUPR_X75439_2559_2589_R	TGGTATATTCGTTAATTAATCTGGCTGCGGA	1303
3019	MUPR_X75439_2490_2514_F	TGGGCTCTTTCTCGCTTAAACACCT	587	MUPR_X75439_2554_2581_R	TCGTTAATTAATCTGGCTGCGGAAGTGA	1112
3020	AROE_NC003923-1674726-1674277_204_232_F	TGATGGCAAGTGGATAGGGTATAATACAG	474	AROE_NC003923-1674726-1674277_309_335_R	TAAGCAATACCTTTACTTGCACCACCT	868
3021	AROE_NC003923-1674726-1674277_207_232_F	TGGCGAGTGGATAGGGTATAATACAG	570	AROE_NC003923-1674726-1674277_311_339_R	TTCATAAGCAATACCTTTACTTGCACCAC	1378
3022	AROE_NC003923-1674726-	TGGCpAAGTpGGATpAGGGTpATpAATpACpAG	572	AROE_NC003923-1674726-1674277_311_335P_R	TAAGCAATACCpTpTpTpACTpTpGCpACpCpAC	867

DOCKET NO.: DIBIS-0083US1 (COUNSEL DOCKET NO: 10593)

	1674277_207_232P_F					
3023	ARCC_NC003923-2725050-2724595_124_155_F	TCTGAAATGAATAGTGATAGAACTGTAGGCAC	398	ARCC_NC003923-2725050-2724595_214_245_R	TCTTCTTCTTTCGTATAAAAAGGACCAATTGG	1137
3024	ARCC_NC003923-2725050-2724595_131_161_F	TGAATAGTGATAGAACTGTAGGCACAATCGT	437	ARCC_NC003923-2725050-2724595_212_242_R	TCTTCTTTCGTATAAAAAGGACCAATTGGTT	1139
3025	ARCC_NC003923-2725050-2724595_131_161_F	TGAATAGTGATAGAACTGTAGGCACAATCGT	437	ARCC_NC003923-2725050-2724595_232_260_R	TGCGCTAATTCTTCAACTTCTTCTTTCGT	1232
3026	PTA_NC003923-628885-629355_231_259_F	TACAATGCTTGTTTATGCTGGTAAAGCAG	177	PTA_NC003923-628885-629355_322_351_R	TGTTCTTGATACACCTGGTTTCGTTTTGAT	1350
3027	PTA_NC003923-628885-629355_231_259_F	TACAATGCTTGTTTATGCTGGTAAAGCAG	177	PTA_NC003923-628885-629355_314_345_R	TGGTACACCTGGTTTCGTTTTGATGATTTGTA	1301
3028	PTA_NC003923-628885-629355_237_263_F	TCTTGTTTATGCTGGTAAAGCAGATGG	418	PTA_NC003923-628885-629355_322_351_R	TGTTCTTGATACACCTGGTTTCGTTTTGAT	1350

[370] Primer pair name codes and reference sequences are shown in Table 3. The primer name code typically represents the gene to which the given primer pair is targeted. The primer pair name may include specific coordinates with respect to a reference sequence defined by an extraction of a section of sequence or defined by a GenBank gi number, or the corresponding complementary sequence of the extraction, or the entire GenBank gi number as indicated by the label "no extraction." Where "no extraction" is indicated for a reference sequence, the coordinates of a primer pair named to the reference sequence are with respect to the GenBank gi listing. Gene abbreviations are shown in bold type in the "Gene Name" column.

[371] To determine the exact primer hybridization coordinates of a given pair of primers on a given bioagent nucleic acid sequence and to determine the sequences, molecular masses and base compositions of an amplification product to be obtained upon amplification of nucleic acid of a known bioagent with known sequence information in the region of interest with a given pair of primers, one with ordinary skill in bioinformatics is capable of obtaining alignments of the primers of the present invention with the GenBank gi number of the relevant nucleic acid sequence of the known bioagent. For example, the reference sequence GenBank gi numbers (Table 3) provide the identities of the sequences which can be obtained from GenBank. Alignments can be done using a bioinformatics tool such as BLASTn provided to the public by NCBI (Bethesda, MD). Alternatively, a relevant GenBank sequence may be downloaded and imported into custom programmed or commercially available bioinformatics programs wherein the alignment can be carried out to determine the primer hybridization coordinates and the sequences, molecular masses and base compositions of the amplification product. For example, to obtain the hybridization coordinates of primer pair number 2095 (SEQ ID NOs: 456:1261), First the forward primer (SEQ ID NO: 456) is subjected to a BLASTn search on the publicly available NCBI BLAST website. "RefSeq_Genomic" is chosen as the BLAST database since the gi numbers refer to genomic sequences. The BLAST query is then performed. Among the top results returned is a match to GenBank gi number 21281729 (Accession Number NC_003923). The result shown below, indicates that the forward primer hybridizes to positions 1530282..1530307 of the genomic sequence of *Staphylococcus aureus* subsp. aureus MW2 (represented by gi number 21281729).

```
Staphylococcus aureus subsp. aureus MW2, complete genome
Length=2820462

 Features in this part of subject sequence:
   Panton-Valentine leukocidin chain F precursor

 Score = 52.0 bits (26),  Expect = 2e-05
 Identities = 26/26 (100%), Gaps = 0/26 (0%)
```

```
Strand=Plus/Plus

Query  1        TGAGCTGCATCAACTGTATTGGATAG  26
                ||||||||||||||||||||||||||
Sbjct  1530282  TGAGCTGCATCAACTGTATTGGATAG  1530307
```

[372] The hybridization coordinates of the reverse primer (SEQ ID NO: 1261) can be determined in a similar manner and thus, the bioagent identifying amplicon can be defined in terms of genomic coordinates. The query/subject arrangement of the result would be presented in Strand = Plus/Minus format because the reverse strand hybridizes to the reverse complement of the genomic sequence. HThe preceding sequence analyses are well known to one with ordinary skill in bioinformatics and thus, Table 3 contains sufficient information to determine the primer hybridization coordinates of any of the primers of Table 2 to the applicable reference sequences described therein.

Table 3: Primer Name Codes and Reference Sequence

Primer name code	Gene Name	Organism	Reference GenBank gi number
16S_EC	16S rRNA (16S ribosomal RNA gene)	*Escherichia coli*	16127994
23S_EC	23S rRNA (23S ribosomal RNA gene)	*Escherichia coli*	16127994
CAPC_BA	capC (capsule biosynthesis gene)	*Bacillus anthracis*	6470151
CYA_BA	cya (cyclic AMP gene)	*Bacillus anthracis*	4894216
DNAK_EC	dnaK (chaperone dnaK gene)	*Escherichia coli*	16127994
GROL_EC	groL (chaperonin groL)	*Escherichia coli*	16127994
HFLB_EC	hflb (cell division protein peptidase ftsH)	*Escherichia coli*	16127994
INFB_EC	infB (protein chain initiation factor infB gene)	*Escherichia coli*	16127994
LEF_BA	lef (lethal factor)	*Bacillus anthracis*	21392688
PAG_BA	pag (protective antigen)	*Bacillus anthracis*	21392688
RPLB_EC	rplB (50S ribosomal protein L2)	*Escherichia coli*	16127994
RPOB_EC	rpoB (DNA-directed RNA polymerase beta chain)	*Escherichia coli*	6127994
RPOC_EC	rpoC (DNA-directed RNA polymerase beta' chain)	*Escherichia coli*	16127994
SP101ET_SPET_11	Artificial Sequence Concatenation comprising: gki (glucose kinase) gtr (glutamine transporter protein) murI (glutamate racemase) mutS (DNA mismatch repair protein) xpt (xanthine phosphoribosyl transferase) yqiL (acetyl-CoA-acetyl transferase) tkt (transketolase)	Artificial Sequence* - partial gene sequences of *Streptococcus pyogenes*	15674250
SSPE_BA	sspE (small acid-soluble spore protein)	*Bacillus anthracis*	30253828
TUFB_EC	tufB (Elongation factor Tu)	*Escherichia coli*	16127994

VALS_EC	valS (Valyl-tRNA synthetase)	*Escherichia coli*	16127994
ASPS_EC	aspS (Aspartyl-tRNA synthetase)	*Escherichia coli*	16127994
CAF1_AF053947	caf1 (capsular protein caf1)	*Yersinia pestis*	2996286
INV_U22457	inv (invasin)	*Yersinia pestis*	1256565
LL_NC003143	*Y. pestis* specific chromosomal genes - difference region	*Yersinia pestis*	16120353
BONTA_X52066	BoNT/A (neurotoxin type A)	*Clostridium botulinum*	40381
MECA_Y14051	mecA methicillin resistance gene	*Staphylococcus aureus*	2791983
TRPE_AY094355	trpE (anthranilate synthase (large component))	*Acinetobacter baumanii*	20853695
RECA_AF251469	recA (recombinase A)	*Acinetobacter baumanii*	9965210
GYRA_AF100557	gyrA (DNA gyrase subunit A)	*Acinetobacter baumanii*	4240540
GYRB_AB008700	gyrB (DNA gyrase subunit B)	*Acinetobacter baumanii*	4514436
WAAA_Z96925	waaA (3-deoxy-D-manno-octulosonic-acid transferase)	*Acinetobacter baumanii*	2765828
CJST_CJ	Artificial Sequence Concatenation comprising: tkt (transketolase) glyA (serine hydroxymethyltransferase) gltA (citrate synthase) aspA (aspartate ammonia lyase) glnA (glutamine synthase) pgm (phosphoglycerate mutase) uncA (ATP synthetase alpha chain)	Artificial Sequence* - partial gene sequences of *Campylobacter jejuni*	15791399
RNASEP_BDP	RNase P (ribonuclease P)	*Bordetella pertussis*	33591275
RNASEP_BKM	RNase P (ribonuclease P)	*Burkholderia mallei*	53723370
RNASEP_BS	RNase P (ribonuclease P)	*Bacillus subtilis*	16077068
RNASEP_CLB	RNase P (ribonuclease P)	*Clostridium perfringens*	18308982
RNASEP_EC	RNase P (ribonuclease P)	*Escherichia coli*	16127994
RNASEP_RKP	RNase P (ribonuclease P)	*Rickettsia prowazekii*	15603881
RNASEP_SA	RNase P (ribonuclease P)	*Staphylococcus aureus*	15922990
RNASEP_VBC	RNase P (ribonuclease P)	*Vibrio cholerae*	15640032
ICD_CXB	icd (isocitrate dehydrogenase)	*Coxiella burnetii*	29732244
IS1111A	multi-locus IS1111A insertion element	*Acinetobacter baumannii*	29732244
OMPA_AY485227	ompA (outer membrane protein A)	*Rickettsia prowazekii*	40287451
OMPB_RKP	ompB (outer membrane protein B)	*Rickettsia prowazekii*	15603881
GLTA_RKP	gltA (citrate synthase)	*Vibrio cholerae*	15603881
TOXR_VBC	toxR (transcription regulator toxR)	*Francisella tularensis*	15640032
ASD_FRT	asd (Aspartate semialdehyde dehydrogenase)	*Francisella tularensis*	56707187
GALE_FRT	galE (UDP-glucose 4-epimerase)	*Shigella flexneri*	56707187
IPAH_SGF	ipaH (invasion plasmid antigen)	*Campylobacter jejuni*	30061571
HUPB_CJ	hupB (DNA-binding protein Hu-beta)	*Coxiella burnetii*	15791399
AB_MLST	Artificial Sequence Concatenation comprising: trpE (anthranilate synthase component	Artificial Sequence* - partial gene	Sequenced in-house (SEQ ID NO: 1444)

	I)) adk (adenylate kinase) mutY (adenine glycosylase) fumC (fumarate hydratase) efp (elongation factor p) ppa (pyrophosphate phospho-hydratase	sequences of *Acinetobacter baumannii*	
MUPR_X75439	**mupR** (mupriocin resistance gene)	*Staphylococcus aureus*	438226
PARC_X95819	**parC** (topoisomerase IV)	*Acinetobacter baumannii*	1212748
SED_M28521	**sed** (enterotoxin D)	*Staphylococcus aureus*	1492109
PLA_AF053945	**pla** (plasminogen activator)	*Yersinia pestis*	2996216
SEJ_AF053140	**sej** (enterotoxin J)	*Staphylococcus aureus*	3372540
GYRA_NC000912	**gyrA** (DNA gyrase subunit A)	*Mycoplasma pneumoniae*	13507739
ACS_NC002516	**acsA** (Acetyl CoA Synthase)	*Pseudomonas aeruginosa*	15595198
ARO_NC002516	**aroE** (shikimate 5-dehydrogenase	*Pseudomonas aeruginosa*	15595198
GUA_NC002516	**guaA** (GMP synthase)	*Pseudomonas aeruginosa*	15595198
MUT_NC002516	**mutL** (DNA mismatch repair protein)	*Pseudomonas aeruginosa*	15595198
NUO_NC002516	**nuoD** (NADH dehydrogenase I chain C, D)	*Pseudomonas aeruginosa*	15595198
PPS_NC002516	**ppsA** (Phosphoenolpyruvate synthase)	*Pseudomonas aeruginosa*	15595198
TRP_NC002516	**trpE** (Anthranilate synthetase component I)	*Pseudomonas aeruginosa*	15595198
OMP2_NC000117	**ompB** (outer membrane protein B)	*Chlamydia trachomatis*	15604717
OMPA_NC000117	**ompA** (outer membrane protein B)	*Chlamydia trachomatis*	15604717
GYRA_NC000117	**gyrA** (DNA gyrase subunit A)	*Chlamydia trachomatis*	15604717
CTXA_NC002505	**ctxA** (Cholera toxin A subunit)	*Vibrio cholerae*	15640032
CTXB_NC002505	**ctxB** (Cholera toxin B subunit)	*Vibrio cholerae*	15640032
FUR_NC002505	**fur** (ferric uptake regulator protein)	*Vibrio cholerae*	15640032
GAPA_NC_002505	**gapA** (glyceraldehyde-3-phosphate dehydrogenase)	*Vibrio cholerae*	15640032
GYRB_NC002505	**gyrB** (DNA gyrase subunit B)	*Vibrio cholerae*	15640032
OMPU_NC002505	**ompU** (outer membrane protein)	*Vibrio cholerae*	15640032
TCPA_NC002505	**tcpA** (toxin-coregulated pilus)	*Vibrio cholerae*	15640032
ASPA_NC002163	**aspA** (aspartate ammonia lyase)	*Campylobacter jejuni*	15791399
GLNA_NC002163	**glnA** (glutamine synthetase)	*Campylobacter jejuni*	15791399
GLTA_NC002163	**gltA** (glutamate synthase)	*Campylobacter jejuni*	15791399
GLYA_NC002163	**glyA** (serine hydroxymethyltransferase)	*Campylobacter jejuni*	15791399
PGM_NC002163	**pgm** (phosphoglyceromutase)	*Campylobacter jejuni*	15791399
TKT_NC002163	**tkt** (transketolase)	*Campylobacter jejuni*	15791399
UNCA_NC002163	**uncA** (ATP synthetase alpha chain)	*Campylobacter jejuni*	15791399
AGR-III_NC003923	**agr-III** (accessory gene regulator-III)	*Staphylococcus aureus*	21281729
ARCC_NC003923	**arcC** (carbamate kinase)	*Staphylococcus aureus*	21281729
AROE_NC003923	**aroE** (shikimate 5-dehydrogenase	*Staphylococcus*	21281729

		aureus	
BSA-A_NC003923	**bsa-a** (glutathione peroxidase)	*Staphylococcus aureus*	21281729
BSA-B_NC003923	**bsa-b** (epidermin biosynthesis protein EpiB)	*Staphylococcus aureus*	21281729
GLPF_NC003923	**glpF** (glycerol transporter)	*Staphylococcus aureus*	21281729
GMK_NC003923	**gmk** (guanylate kinase)	*Staphylococcus aureus*	21281729
MECI-R_NC003923	**mecR1** (truncated methicillin resistance protein)	*Staphylococcus aureus*	21281729
PTA_NC003923	**pta** (phosphate acetyltransferase)	*Staphylococcus aureus*	21281729
PVLUK_NC003923	**pvluk** (Panton-Valentine leukocidin chain F precursor)	*Staphylococcus aureus*	21281729
SA442_NC003923	**sa442** gene	*Staphylococcus aureus*	21281729
SEA_NC003923	**sea** (staphylococcal enterotoxin A precursor)	*Staphylococcus aureus*	21281729
SEC_NC003923	**sec4** (enterotoxin type C precursor)	*Staphylococcus aureus*	21281729
TPI_NC003923	**tpi** (triosephosphate isomerase)	*Staphylococcus aureus*	21281729
YQI_NC003923	**yqi** (acetyl-CoA C-acetyltransferase homologue)	*Staphylococcus aureus*	21281729
GALE_AF513299	**galE** (galactose epimerase)	*Francisella tularensis*	23506418
VVHA_NC004460	**vVhA** (cytotoxin, cytolysin precursor)	*Vibrio vulnificus*	27366463
TDH_NC004605	**tdh** (thermostable direct hemolysin A)	*Vibrio parahaemolyticus*	28899855
AGR-II_NC002745	**agr-II** (accessory gene regulator-II)	*Staphylococcus aureus*	29165615
PARC_NC003997	**parC** (topoisomerase IV)	*Bacillus anthracis*	30260195
GYRA_AY291534	**gyrA** (DNA gyrase subunit A)	*Bacillus anthracis*	31323274
AGR-I_AJ617706	**agr-I** (accessory gene regulator-I)	*Staphylococcus aureus*	46019543
AGR-IV_AJ617711	**agr-IV** (accessory gene regulator-III)	*Staphylococcus aureus*	46019563
BLAZ_NC002952	**blaZ** (beta lactamase III)	*Staphylococcus aureus*	49482253
ERMA_NC002952	**ermA** (rRNA methyltransferase A)	*Staphylococcus aureus*	49482253
ERMB_Y13600	**ermB** (rRNA methyltransferase B)	*Staphylococcus aureus*	49482253
SEA-SEE_NC002952	**sea** (staphylococcal enterotoxin A precursor)	*Staphylococcus aureus*	49482253
SEA-SEE_NC002952	**sea** (staphylococcal enterotoxin A precursor)	*Staphylococcus aureus*	49482253
SEE_NC002952	**sea** (staphylococcal enterotoxin A precursor)	*Staphylococcus aureus*	49482253
SEH_NC002953	**seh** (staphylococcal enterotoxin H)	*Staphylococcus aureus*	49484912
ERMC_NC005908	**ermC** (rRNA methyltransferase C)	*Staphylococcus aureus*	49489772
MUTS_AY698802	**mutS** (DNA mismatch repair protein)	*Shigella boydii*	52698233
NUC_NC002758	**nuc** (staphylococcal nuclease)	*Staphylococcus aureus*	57634611
SEB_NC002758	**seb** (enterotoxin type B precursor)	*Staphylococcus aureus*	57634611
SEG_NC002758	**seg** (staphylococcal enterotoxin G)	*Staphylococcus aureus*	57634611
SEI_NC002758	**sei** (staphylococcal enterotoxin I)	*Staphylococcus aureus*	57634611
TSST_NC002758	**tsst** (toxic shock syndrome toxin-1)	*Staphylococcus aureus*	57634611
TUFB_NC002758	**tufB** (Elongation factor Tu)	*Staphylococcus aureus*	57634611

[373] Note: artificial reference sequences represent concatenations of partial gene extractions from the indicated reference gi number. Partial sequences were used to create the concatenated sequence because complete gene sequences were not necessary for primer design.

Example 2: Sample Preparation and PCR

[374] Genomic DNA was prepared from samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols.

[375] All PCR reactions were assembled in 50 μL reaction volumes in a 96-well microtiter plate format using a Packard MPII liquid handling robotic platform and M.J. Dyad thermocyclers (MJ research, Waltham, MA) or Eppendorf Mastercycler thermocyclers (Eppendorf, Westbury, NY). The PCR reaction mixture consisted of 4 units of Amplitaq Gold, 1x buffer II (Applied Biosystems, Foster City, CA), 1.5 mM $MgCl_2$, 0.4 M betaine, 800 μM dNTP mixture and 250 nM of each primer. The following typical PCR conditions were used: 95°C for 10 min followed by 8 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C 30 seconds with the 48°C annealing temperature increasing 0.9°C with each of the eight cycles. The PCR was then continued for 37 additional cycles of 95°C for 15 seconds, 56°C for 20 seconds, and 72°C 20 seconds.

Example 3: Purification of PCR Products for Mass Spectrometry with Ion Exchange Resin-Magnetic Beads

[376] For solution capture of nucleic acids with ion exchange resin linked to magnetic beads, 25 μl of a 2.5 mg/mL suspension of BioClone amine terminated superparamagnetic beads were added to 25 to 50 μl of a PCR (or RT-PCR) reaction containing approximately 10 pM of a typical PCR amplification product. The above suspension was mixed for approximately 5 minutes by vortexing or pipetting, after which the liquid was removed after using a magnetic separator. The beads containing bound PCR amplification product were then washed three times with 50mM ammonium bicarbonate/50% MeOH or 100mM ammonium bicarbonate/50% MeOH, followed by three more washes with 50% MeOH. The bound PCR amplicon was eluted with a solution of 25mM piperidine, 25mM imidazole, 35% MeOH which included peptide calibration standards.

Example 4: Mass Spectrometry and Base Composition Analysis

[377] The ESI-FTICR mass spectrometer is based on a Bruker Daltonics (Billerica, MA) Apex II 70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer that employs an actively shielded 7 Tesla superconducting magnet. The active shielding constrains the majority of the fringing magnetic field from the superconducting magnet to a relatively small volume. Thus,

components that might be adversely affected by stray magnetic fields, such as CRT monitors, robotic components, and other electronics, can operate in close proximity to the FTICR spectrometer. All aspects of pulse sequence control and data acquisition were performed on a 600 MHz Pentium II data station running Bruker's Xmass software under Windows NT 4.0 operating system. Sample aliquots, typically 15 μl, were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC) triggered by the FTICR data station. Samples were injected directly into a 10 μl sample loop integrated with a fluidics handling system that supplies the 100 μl /hr flow rate to the ESI source. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned approximately 1.5 cm from the metalized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary was biased at 6000 V relative to the ESI needle during data acquisition. A counter-current flow of dry N_2 was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed. Ionization duty cycles greater than 99% were achieved by simultaneously accumulating ions in the external ion reservoir during ion detection. Each detection event consisted of 1M data points digitized over 2.3 s. To improve the signal-to-noise ratio (S/N), 32 scans were co-added for a total data acquisition time of 74 s.

[378] The ESI-TOF mass spectrometer is based on a Bruker Daltonics MicroTOF™. Ions from the ESI source undergo orthogonal ion extraction and are focused in a reflectron prior to detection. The TOF and FTICR are equipped with the same automated sample handling and fluidics described above. Ions are formed in the standard MicroTOF™ ESI source that is equipped with the same off-axis sprayer and glass capillary as the FTICR ESI source. Consequently, source conditions were the same as those described above. External ion accumulation was also employed to improve ionization duty cycle during data acquisition. Each detection event on the TOF was comprised of 75,000 data points digitized over 75 μs.

[379] The sample delivery scheme allows sample aliquots to be rapidly injected into the electrospray source at high flow rate and subsequently be electrosprayed at a much lower flow rate for improved ESI sensitivity. Prior to injecting a sample, a bolus of buffer was injected at a high flow rate to rinse the transfer line and spray needle to avoid sample contamination/carryover. Following the rinse step, the autosampler injected the next sample and the flow rate was switched to low flow. Following a brief equilibration delay, data acquisition commenced. As spectra were co-added, the autosampler continued rinsing the syringe and picking up buffer to rinse the injector and sample transfer line. In general, two syringe rinses and one injector rinse were required to minimize sample carryover. During a

routine screening protocol a new sample mixture was injected every 106 seconds. More recently a fast wash station for the syringe needle has been implemented which, when combined with shorter acquisition times, facilitates the acquisition of mass spectra at a rate of just under one spectrum/minute.

[380] Raw mass spectra were post-calibrated with an internal mass standard and deconvoluted to monoisotopic molecular masses. Unambiguous base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Quantitative results are obtained by comparing the peak heights with an internal PCR calibration standard present in every PCR well at 500 molecules per well. Calibration methods are commonly owned and disclosed in U.S. Provisional Patent Application Serial No. 60/545,425 which is incorporated herein by reference in entirety.

Example 5: *De Novo* Determination of Base Composition of Amplification Products using Molecular Mass Modified Deoxynucleotide Triphosphates

[381] Because the molecular masses of the four natural nucleobases have a relatively narrow molecular mass range (A = 313.058, G = 329.052, C = 289.046, T = 304.046 – See Table 4), a persistent source of ambiguity in assignment of base composition can occur as follows: two nucleic acid strands having different base composition may have a difference of about 1 Da when the base composition difference between the two strands is G $\leftrightarrow$ A (-15.994) combined with C $\leftrightarrow$ T (+15.000). For example, one 99-mer nucleic acid strand having a base composition of $A_{27}G_{30}C_{21}T_{21}$ has a theoretical molecular mass of 30779.058 while another 99-mer nucleic acid strand having a base composition of $A_{26}G_{31}C_{22}T_{20}$ has a theoretical molecular mass of 30780.052. A 1 Da difference in molecular mass may be within the experimental error of a molecular mass measurement and thus, the relatively narrow molecular mass range of the four natural nucleobases imposes an uncertainty factor.

[382] The present invention provides for a means for removing this theoretical 1 Da uncertainty factor through amplification of a nucleic acid with one mass-tagged nucleobase and three natural nucleobases. The term "nucleobase" as used herein is synonymous with other terms in use in the art including "nucleotide," "deoxynucleotide," "nucleotide residue," "deoxynucleotide residue," "nucleotide triphosphate (NTP)," or deoxynucleotide triphosphate (dNTP).

[383] Addition of significant mass to one of the 4 nucleobases (dNTPs) in an amplification reaction, or in the primers themselves, will result in a significant difference in mass of the resulting amplification product (significantly greater than 1 Da) arising from ambiguities arising from the G $\leftrightarrow$ A combined with C $\leftrightarrow$ T event (Table 4). Thus, the same the G $\leftrightarrow$ A (-15.994) event combined with 5-Iodo-C $\leftrightarrow$ T (-110.900) event would result in a molecular mass difference of 126.894. If the molecular mass of the

base composition $A_{27}G_{30}$ **5-Iodo-C_{21}**T_{21} (33422.958) is compared with $A_{26}G_{31}$**5-Iodo-C_{22}**T_{20}, (33549.852) the theoretical molecular mass difference is +126.894. The experimental error of a molecular mass measurement is not significant with regard to this molecular mass difference. Furthermore, the only base composition consistent with a measured molecular mass of the 99-mer nucleic acid is $A_{27}G_{30}$**5-Iodo-C_{21}**T_{21}. In contrast, the analogous amplification without the mass tag has 18 possible base compositions.

Table 4: Molecular Masses of Natural Nucleobases and the Mass-Modified Nucleobase 5-Iodo-C and Molecular Mass Differences Resulting from Transitions

Nucleobase	Molecular Mass	Transition	Δ Molecular Mass
A	313.058	A-->T	-9.012
A	313.058	A-->C	-24.012
A	313.058	A-->5-Iodo-C	101.888
A	313.058	A-->G	15.994
T	304.046	T-->A	9.012
T	304.046	T-->C	-15.000
T	304.046	T-->5-Iodo-C	110.900
T	304.046	T-->G	25.006
C	289.046	C-->A	24.012
C	289.046	C-->T	15.000
C	289.046	C-->G	40.006
5-Iodo-C	414.946	5-Iodo-C-->A	-101.888
5-Iodo-C	414.946	5-Iodo-C-->T	-110.900
5-Iodo-C	414.946	5-Iodo-C-->G	-85.894
G	329.052	G-->A	-15.994
G	329.052	G-->T	-25.006
G	329.052	G-->C	-40.006
G	329.052	G-->5-Iodo-C	85.894

[384] Mass spectra of bioagent-identifying amplicons were analyzed independently using a maximum-likelihood processor, such as is widely used in radar signal processing. This processor, referred to as GenX, first makes maximum likelihood estimates of the input to the mass spectrometer for each primer by running matched filters for each base composition aggregate on the input data. This includes the GenX response to a calibrant for each primer.

[385] The algorithm emphasizes performance predictions culminating in probability-of-detection versus probability-of-false-alarm plots for conditions involving complex backgrounds of naturally occurring organisms and environmental contaminants. Matched filters consist of *a priori* expectations of signal values given the set of primers used for each of the bioagents. A genomic sequence database is

used to define the mass base count matched filters. The database contains the sequences of known bacterial bioagents and includes threat organisms as well as benign background organisms. The latter is used to estimate and subtract the spectral signature produced by the background organisms. A maximum likelihood detection of known background organisms is implemented using matched filters and a running-sum estimate of the noise covariance. Background signal strengths are estimated and used along with the matched filters to form signatures which are then subtracted. The maximum likelihood process is applied to this "cleaned up" data in a similar manner employing matched filters for the organisms and a running-sum estimate of the noise-covariance for the cleaned up data.

[386] The amplitudes of all base compositions of bioagent-identifying amplicons for each primer are calibrated and a final maximum likelihood amplitude estimate per organism is made based upon the multiple single primer estimates. Models of all system noise are factored into this two-stage maximum likelihood calculation. The processor reports the number of molecules of each base composition contained in the spectra. The quantity of amplification product corresponding to the appropriate primer set is reported as well as the quantities of primers remaining upon completion of the amplification reaction.

[387] Base count blurring can be carried out as follows. "Electronic PCR" can be conducted on nucleotide sequences of the desired bioagents to obtain the different expected base counts that could be obtained for each primer pair. See for example, ncbi.nlm.nih.gov/sutils/e-pcr/; Schuler, *Genome Res.* 7:541-50, 1997. In one illustrative embodiment, one or more spreadsheets, such as Microsoft Excel workbooks contain a plurality of worksheets. First in this example, there is a worksheet with a name similar to the workbook name; this worksheet contains the raw electronic PCR data. Second, there is a worksheet named "filtered bioagents base count" that contains bioagent name and base count; there is a separate record for each strain after removing sequences that are not identified with a genus and species and removing all sequences for bioagents with less than 10 strains. Third, there is a worksheet, "Sheet1" that contains the frequency of substitutions, insertions, or deletions for this primer pair. This data is generated by first creating a pivot table from the data in the "filtered bioagents base count" worksheet and then executing an Excel VBA macro. The macro creates a table of differences in base counts for bioagents of the same species, but different strains. One of ordinary skill in the art may understand additional pathways for obtaining similar table differences without undo experimentation.

[388] Application of an exemplary script, involves the user defining a threshold that specifies the fraction of the strains that are represented by the reference set of base counts for each bioagent. The reference set of base counts for each bioagent may contain as many different base counts as are needed

to meet or exceed the threshold. The set of reference base counts is defined by taking the most abundant strain's base type composition and adding it to the reference set and then the next most abundant strain's base type composition is added until the threshold is met or exceeded. The current set of data was obtained using a threshold of 55%, which was obtained empirically.

[389] For each base count not included in the reference base count set for that bioagent, the script then proceeds to determine the manner in which the current base count differs from each of the base counts in the reference set. This difference may be represented as a combination of substitutions, Si=Xi, and insertions, Ii=Yi, or deletions, Di=Zi. If there is more than one reference base count, then the reported difference is chosen using rules that aim to minimize the number of changes and, in instances with the same number of changes, minimize the number of insertions or deletions. Therefore, the primary rule is to identify the difference with the minimum sum (Xi+Yi) or (Xi+Zi), *e.g.*, one insertion rather than two substitutions. If there are two or more differences with the minimum sum, then the one that will be reported is the one that contains the most substitutions.

[390] Differences between a base count and a reference composition are categorized as one, two, or more substitutions, one, two, or more insertions, one, two, or more deletions, and combinations of substitutions and insertions or deletions. The different classes of nucleobase changes and their probabilities of occurrence have been delineated in U.S. Patent Application Publication No. 2004209260 (U.S. Application Serial No. 10/418,514) which is incorporated herein by reference in entirety.

Example 6: Use of Broad Range Survey and Division Wide Primer Pairs for Identification of Bacteria in an Epidemic Surveillance Investigation

[391] This investigation employed a set of 16 primer pairs which is herein designated the "surveillance primer set" and comprises broad range survey primer pairs, division wide primer pairs and a single *Bacillus* clade primer pair. The surveillance primer set is shown in Table 5 and consists of primer pairs originally listed in Table 2. This surveillance set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row. Primer pair 449 (non-T modified) has been modified twice. Its predecessors are primer pairs 70 and 357, displayed below in the same row. Primer pair 360 has also been modified twice and its predecessors are primer pairs 17 and 118.

Table 5: Bacterial Primer Pairs of the Surveillance Primer Set

Primer Pair	Forward Primer Name	Forward Primer	Reverse Primer Name	Reverse Primer	Target Gene

No.		(SEQ ID NO:)		(SEQ ID NO:)	
346	16S_EC_713_732_TMOD_F	202	16S_EC_789_809_TMOD_R	1110	16S rRNA
10	16S_EC_713_732_F	21	16S_EC_789_809	798	16S rRNA
347	16S_EC_785_806_TMOD_F	560	16S_EC_880_897_TMOD_R	1278	16S rRNA
11	16S_EC_785_806_F	118	16S_EC_880_897_R	830	16S rRNA
348	16S_EC_960_981_TMOD_F	706	16S_EC_1054_1073_TMOD_R	895	16S rRNA
14	16S_EC_960_981_F	672	16S_EC_1054_1073_R	735	16S rRNA
349	23S_EC_1826_1843_TMOD_F	401	23S_EC_1906_1924_TMOD_R	1156	23S rRNA
16	23S_EC_1826_1843_F	80	23S_EC_1906_1924_R	805	23S rRNA
352	INFB_EC_1365_1393_TMOD_F	687	INFB_EC_1439_1467_TMOD_R	1411	infB
34	INFB_EC_1365_1393_F	524	INFB_EC_1439_1467_R	1248	infB
354	RPOC_EC_2218_2241_TMOD_F	405	RPOC_EC_2313_2337_TMOD_R	1072	rpoC
52	RPOC_EC_2218_2241_F	81	RPOC_EC_2313_2337_R	790	rpoC
355	SSPE_BA_115_137_TMOD_F	255	SSPE_BA_197_222_TMOD_R	1402	sspE
58	SSPE_BA_115_137_F	45	SSPE_BA_197_222_R	1201	sspE
356	RPLB_EC_650_679_TMOD_F	232	RPLB_EC_739_762_TMOD_R	592	rplB
66	RPLB_EC_650_679_F	98	RPLB_EC_739_762_R	999	rplB
358	VALS_EC_1105_1124_TMOD_F	385	VALS_EC_1195_1218_TMOD_R	1093	valS
71	VALS_EC_1105_1124_F	77	VALS_EC_1195_1218_R	795	valS
359	RPOB_EC_1845_1866_TMOD_F	659	RPOB_EC_1909_1929_TMOD_R	1250	rpoB
72	RPOB_EC_1845_1866_F	233	RPOB_EC_1909_1929_R	825	rpoB
360	23S_EC_2646_2667_TMOD_F	409	23S_EC_2745_2765_TMOD_R	1434	23S rRNA
118	23S_EC_2646_2667_F	84	23S_EC_2745_2765_R	1389	23S rRNA
17	23S_EC_2645_2669_F	408	23S_EC_2744_2761_R	1252	23S rRNA
361	16S_EC_1090_1111_2_TMOD_F	697	16S_EC_1175_1196_TMOD_R	1398	16S rRNA
3	16S_EC_1090_1111_2_F	651	16S_EC_1175_1196_R	1159	16S rRNA
362	RPOB_EC_3799_3821_TMOD_F	581	RPOB_EC_3862_3888_TMOD_R	1325	rpoB
289	RPOB_EC_3799_3821_F	124	RPOB_EC_3862_3888_R	840	rpoB
363	RPOC_EC_2146_2174_TMOD_F	284	RPOC_EC_2227_2245_TMOD_R	898	rpoC
290	RPOC_EC_2146_2174_F	52	RPOC_EC_2227_2245_R	736	rpoC
367	TUFB_EC_957_979_TMOD_F	308	TUFB_EC_1034_1058_TMOD_R	1276	tufB
293	TUFB_EC_957_979_F	55	TUFB_EC_1034_1058_R	829	tufB
449	RPLB_EC_690_710_F	309	RPLB_EC_737_758_R	1336	rplB
357	RPLB_EC_688_710_TMOD_F	296	RPLB_EC_736_757_TMOD_R	1337	rplB
67	RPLB_EC_688_710_F	54	RPLB_EC_736_757_R	842	rplB

[392] The 16 primer pairs of the surveillance set are used to produce bioagent identifying amplicons whose base compositions are sufficiently different amongst all known bacteria at the species level to identify, at a reasonable confidence level, any given bacterium at the species level. As shown in Tables 6A-E, common respiratory bacterial pathogens can be distinguished by the base compositions of bioagent identifying amplicons obtained using the 16 primer pairs of the surveillance set. In some cases, triangulation identification improves the confidence level for species assignment. For example, nucleic acid from *Streptococcus pyogenes* can be amplified by nine of the sixteen surveillance primer pairs and *Streptococcus pneumoniae* can be amplified by ten of the sixteen surveillance primer pairs. The base

compositions of the bioagent identifying amplicons are identical for only one of the analogous bioagent identifying amplicons and differ in all of the remaining analogous bioagent identifying amplicons by up to four bases per bioagent identifying amplicon. The resolving power of the surveillance set was confirmed by determination of base compositions for 120 isolates of respiratory pathogens representing 70 different bacterial species and the results indicated that natural variations (usually only one or two base substitutions per bioagent identifying amplicon) amongst multiple isolates of the same species did not prevent correct identification of major pathogenic organisms at the species level.

[393] *Bacillus anthracis* is a well known biological warfare agent which has emerged in domestic terrorism in recent years. Since it was envisioned to produce bioagent identifying amplicons for identification of *Bacillus anthracis*, additional drill-down analysis primers were designed to target genes present on virulence plasmids of *Bacillus anthracis* so that additional confidence could be reached in positive identification of this pathogenic organism. Three drill-down analysis primers were designed and are listed in Tables 2 and 6. In Table 6, the drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

Table 6: Drill-Down Primer Pairs for Confirmation of Identification of *Bacillus anthracis*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
350	CAPC_BA_274_303_TMOD_F	476	CAPC_BA_349_376_TMOD_R	1314	capC
24	CAPC_BA_274_303_F	109	CAPC_BA_349_376_R	837	capC
351	CYA_BA_1353_1379_TMOD_F	355	CYA_BA_1448_1467_TMOD_R	1423	cyA
30	CYA_BA_1353_1379_F	64	CYA_BA_1448_1467_R	1342	cyA
353	LEF_BA_756_781_TMOD_F	220	LEF_BA_843_872_TMOD_R	1394	lef
37	LEF_BA_756_781_F	26	LEF_BA_843_872_R	1135	lef

[394] Phylogenetic coverage of bacterial space of the sixteen surveillance primers of Table 5 and the three *Bacillus* anthracis drill-down primers of Table 6 is shown in Figure 3 which lists common pathogenic bacteria. Figure 3 is not meant to be comprehensive in illustrating all species identified by the primers. Only pathogenic bacteria are listed as representative examples of the bacterial species that can be identified by the primers and methods of the present invention. Nucleic acid of groups of bacteria enclosed within the polygons of Figure 3 can be amplified to obtain bioagent identifying amplicons using the primer pair numbers listed in the upper right hand corner of each polygon. Primer coverage for polygons within polygons is additive. As an illustrative example, bioagent identifying amplicons can be obtained for *Chlamydia trachomatis* by amplification with, for example, primer pairs 346-349, 360 and

361, but not with any of the remaining primers of the surveillance primer set. On the other hand, bioagent identifying amplicons can be obtained from nucleic acid originating from *Bacillus anthracis* (located within 5 successive polygons) using, for example, any of the following primer pairs: 346-349, 360, 361 (base polygon), 356, 449 (second polygon), 352 (third polygon), 355 (fourth polygon), 350, 351 and 353 (fifth polygon). Multiple coverage of a given organism with multiple primers provides for increased confidence level in identification of the organism as a result of enabling broad triangulation identification.

[395] In Tables 7A-E, base compositions of respiratory pathogens for primer target regions are shown. Two entries in a cell, represent variation in ribosomal DNA operons. The most predominant base composition is shown first and the minor (frequently a single operon) is indicated by an asterisk (*). Entries with NO DATA mean that the primer would not be expected to prime this species due to mismatches between the primer and target region, as determined by theoretical PCR.

Table 7A – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 346, 347 and 348

Organism	Strain	Primer 346 [A G C T]	Primer 347 [A G C T]	Primer 348 [A G C T]
Klebsiella pneumoniae	MGH78578	[29 32 25 13] [29 31 25 13]*	[23 38 28 26] [23 37 28 26]*	[26 32 28 30] [26 31 28 30]*
Yersinia pestis	CO-92 Biovar Orientalis	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [30 30 27 29]*
Yersinia pestis	KIM5 P12 (Biovar Mediaevalis)	[29 32 25 13]	[22 39 28 26]	[29 30 28 29]
Yersinia pestis	91001	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [30 30 27 29]*
Haemophilus influenzae	KW20	[28 31 23 17]	[24 37 25 27]	[29 30 28 29]
Pseudomonas aeruginosa	PAO1	[30 31 23 15]	[26 36 29 24] [27 36 29 23]*	[26 32 29 29]
Pseudomonas fluorescens	Pf0-1	[30 31 23 15]	[26 35 29 25]	[28 31 28 29]
Pseudomonas putida	KT2440	[30 31 23 15]	[28 33 27 27]	[27 32 29 28]
Legionella pneumophila	Philadelphia-1	[30 30 24 15]	[33 33 23 27]	[29 28 28 31]
Francisella tularensis	schu 4	[32 29 22 16]	[28 38 26 26]	[25 32 28 31]
Bordetella pertussis	Tohama I	[30 29 24 16]	[23 37 30 24]	[30 32 30 26]
Burkholderia cepacia	J2315	[29 29 27 14]	[27 32 26 29]	[27 36 31 24] [20 42 35 19]*
Burkholderia pseudomallei	K96243	[29 29 27 14]	[27 32 26 29]	[27 36 31 24]
Neisseria gonorrhoeae	FA 1090, ATCC 700825	[29 28 24 18]	[27 34 26 28]	[24 36 29 27]
Neisseria meningitidis	MC58 (serogroup B)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Neisseria meningitidis	serogroup C, FAM18	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Neisseria meningitidis	Z2491 (serogroup A)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
Chlamydophila pneumoniae	TW-183	[31 27 22 19]	NO DATA	[32 27 27 29]

Chlamydophila pneumoniae	AR39	[31 27 22 19]	NO DATA	[32 27 27 29]
Chlamydophila pneumoniae	CWL029	[31 27 22 19]	NO DATA	[32 27 27 29]
Chlamydophila pneumoniae	J138	[31 27 22 19]	NO DATA	[32 27 27 29]
Corynebacterium diphtheriae	NCTC13129	[29 34 21 15]	[22 38 31 25]	[22 33 25 34]
Mycobacterium avium	k10	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium avium	104	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium tuberculosis	CSU#93	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium tuberculosis	CDC 1551	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycobacterium tuberculosis	H37Rv (lab strain)	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
Mycoplasma pneumoniae	M129	[31 29 19 20]	NO DATA	NO DATA
Staphylococcus aureus	MRSA252	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [29 31 30 29]*
Staphylococcus aureus	MSSA476	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
Staphylococcus aureus	COL	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
Staphylococcus aureus	Mu50	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
Staphylococcus aureus	MW2	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
Staphylococcus aureus	N315	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
Staphylococcus aureus	NCTC 8325	[27 30 21 21]	[25 35 30 26] [25 35 31 26]*	[30 29 30 29] [30 29 29 30]
Streptococcus agalactiae	NEM316	[26 32 23 18]	[24 36 31 25] [24 36 30 26]*	[25 32 29 30]
Streptococcus equi	NC_002955	[26 32 23 18]	[23 37 31 25]	[29 30 25 32]
Streptococcus pyogenes	MGAS8232	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pyogenes	MGAS315	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pyogenes	SSI-1	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pyogenes	MGAS10394	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pyogenes	Manfredo (M5)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pyogenes	SF370 (M1)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
Streptococcus pneumoniae	670	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
Streptococcus pneumoniae	R6	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
Streptococcus pneumoniae	TIGR4	[26 32 23 18]	[25 35 28 28]	[25 32 30 29]
Streptococcus gordonii	NCTC7868	[25 33 23 18]	[24 36 31 25]	[25 31 29 31]
Streptococcus mitis	NCTC 12261	[26 32 23 18]	[25 35 30 26]	[25 32 29 30] [24 31 35 29]*
Streptococcus mutans	UA159	[24 32 24 19]	[25 37 30 24]	[28 31 26 31]

Table 7B – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 349, 360, and 356

Organism	Strain	Primer 349	Primer 360	Primer 356

		[A G C T]	[A G C T]	[A G C T]
Klebsiella pneumoniae	MGH78578	[25 31 25 22]	[33 37 25 27]	NO DATA
Yersinia pestis	CO-92 Biovar Orientalis	[25 31 27 20] [25 32 26 20]*	[34 35 25 28]	NO DATA
Yersinia pestis	KIM5 P12 (Biovar Mediaevalis)	[25 31 27 20] [25 32 26 20]*	[34 35 25 28]	NO DATA
Yersinia pestis	91001	[25 31 27 20]	[34 35 25 28]	NO DATA
Haemophilus influenzae	KW20	[28 28 25 20]	[32 38 25 27]	NO DATA
Pseudomonas aeruginosa	PAO1	[24 31 26 20]	[31 36 27 27] [31 36 27 28]*	NO DATA
Pseudomonas fluorescens	Pf0-1	NO DATA	[30 37 27 28] [30 37 27 28]	NO DATA
Pseudomonas putida	KT2440	[24 31 26 20]	[30 37 27 28]	NO DATA
Legionella pneumophila	Philadelphia-1	[23 30 25 23]	[30 39 29 24]	NO DATA
Francisella tularensis	schu 4	[26 31 25 19]	[32 36 27 27]	NO DATA
Bordetella pertussis	Tohama I	[21 29 24 18]	[33 36 26 27]	NO DATA
Burkholderia cepacia	J2315	[23 27 22 20]	[31 37 28 26]	NO DATA
Burkholderia pseudomallei	K96243	[23 27 22 20]	[31 37 28 26]	NO DATA
Neisseria gonorrhoeae	FA 1090, ATCC 700825	[24 27 24 17]	[34 37 25 26]	NO DATA
Neisseria meningitidis	MC58 (serogroup B)	[25 27 22 18]	[34 37 25 26]	NO DATA
Neisseria meningitidis	serogroup C, FAM18	[25 26 23 18]	[34 37 25 26]	NO DATA
Neisseria meningitidis	Z2491 (serogroup A)	[25 26 23 18]	[34 37 25 26]	NO DATA
Chlamydophila pneumoniae	TW-183	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila pneumoniae	AR39	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila pneumoniae	CWL029	[30 28 27 18]	NO DATA	NO DATA
Chlamydophila pneumoniae	J138	[30 28 27 18]	NO DATA	NO DATA
Corynebacterium diphtheriae	NCTC13129	NO DATA	[29 40 28 25]	NO DATA
Mycobacterium avium	k10	NO DATA	[33 35 32 22]	NO DATA
Mycobacterium avium	104	NO DATA	[33 35 32 22]	NO DATA
Mycobacterium tuberculosis	CSU#93	NO DATA	[30 36 34 22]	NO DATA
Mycobacterium tuberculosis	CDC 1551	NO DATA	[30 36 34 22]	NO DATA
Mycobacterium tuberculosis	H37Rv (lab strain)	NO DATA	[30 36 34 22]	NO DATA
Mycoplasma pneumoniae	M129	[28 30 24 19]	[34 31 29 28]	NO DATA
Staphylococcus aureus	MRSA252	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus aureus	MSSA476	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus aureus	COL	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus aureus	Mu50	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus aureus	MW2	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus aureus	N315	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
Staphylococcus	NCTC 8325	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]

aureus				
Streptococcus agalactiae	NEM316	[28 31 22 20]	[33 37 24 28]	[37 30 28 26]
Streptococcus equi	NC_002955	[28 31 23 19]	[33 38 24 27]	[37 31 28 25]
Streptococcus pyogenes	MGAS8232	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	MGAS315	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	SSI-1	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	MGAS10394	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	Manfredo (M5)	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
Streptococcus pyogenes	SF370 (M1)	[28 31 23 19] [28 31 22 20]*	[33 37 24 28]	[38 31 29 23]
Streptococcus pneumoniae	670	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus pneumoniae	R6	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus pneumoniae	TIGR4	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
Streptococcus gordonii	NCTC7868	[28 32 23 20]	[34 36 24 28]	[36 31 29 25]
Streptococcus mitis	NCTC 12261	[28 31 22 20] [29 30 22 20]*	[34 36 24 28]	[37 30 29 25]
Streptococcus mutans	UA159	[26 32 23 22]	[34 37 24 27]	NO DATA

Table 7C – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 449, 354, and 352

Organism	Strain	Primer 449 [A G C T]	Primer 354 [A G C T]	Primer 352 [A G C T]
Klebsiella pneumoniae	MGH78578	NO DATA	[27 33 36 26]	NO DATA
Yersinia pestis	CO-92 Biovar Orientalis	NO DATA	[29 31 33 29]	[32 28 20 25]
Yersinia pestis	KIM5 P12 (Biovar Mediaevalis)	NO DATA	[29 31 33 29]	[32 28 20 25]
Yersinia pestis	91001	NO DATA	[29 31 33 29]	NO DATA
Haemophilus influenzae	KW20	NO DATA	[30 29 31 32]	NO DATA
Pseudomonas aeruginosa	PAO1	NO DATA	[26 33 39 24]	NO DATA
Pseudomonas fluorescens	Pf0-1	NO DATA	[26 33 34 29]	NO DATA
Pseudomonas putida	KT2440	NO DATA	[25 34 36 27]	NO DATA
Legionella pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella tularensis	schu 4	NO DATA	[33 32 25 32]	NO DATA
Bordetella pertussis	Tohama I	NO DATA	[26 33 39 24]	NO DATA
Burkholderia cepacia	J2315	NO DATA	[25 37 33 27]	NO DATA
Burkholderia pseudomallei	K96243	NO DATA	[25 37 34 26]	NO DATA
Neisseria gonorrhoeae	FA 1090, ATCC 700825	[17 23 22 10]	[29 31 32 30]	NO DATA
Neisseria meningitidis	MC58 (serogroup B)	NO DATA	[29 30 32 31]	NO DATA
Neisseria meningitidis	serogroup C, FAM18	NO DATA	[29 30 32 31]	NO DATA

Neisseria meningitidis	Z2491 (serogroup A)	NO DATA	[29 30 32 31]	NO DATA
Chlamydophila pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	AR39	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium diphtheriae	NCTC13129	NO DATA	NO DATA	NO DATA
Mycobacterium avium	k10	NO DATA	NO DATA	NO DATA
Mycobacterium avium	104	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	CSU#93	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	CDC 1551	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
Mycoplasma pneumoniae	M129	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MRSA252	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus aureus	MSSA476	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus aureus	COL	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
Staphylococcus aureus	Mu50	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus aureus	MW2	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus aureus	N315	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
Staphylococcus aureus	NCTC 8325	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
Streptococcus agalactiae	NEM316	[22 20 19 14]	[26 31 27 38]	[29 26 22 28]
Streptococcus equi	NC_002955	[22 21 19 13]	NO DATA	NO DATA
Streptococcus pyogenes	MGAS8232	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pyogenes	MGAS315	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pyogenes	SSI-1	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pyogenes	MGAS10394	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pyogenes	Manfredo (M5)	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pyogenes	SF370 (M1)	[23 21 19 12]	[24 32 30 36]	NO DATA
Streptococcus pneumoniae	670	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
Streptococcus pneumoniae	R6	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
Streptococcus pneumoniae	TIGR4	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
Streptococcus gordonii	NCTC7868	[21 21 19 14]	NO DATA	[29 26 22 28]
Streptococcus mitis	NCTC 12261	[22 20 19 14]	[26 30 32 34]	NO DATA
Streptococcus mutans	UA159	NO DATA	NO DATA	NO DATA

Table 7D – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 355, 358, and 359

Organism	Strain	Primer 355 [A G C T]	Primer 358 [A G C T]	Primer 359 [A G C T]
Klebsiella pneumoniae	MGH78578	NO DATA	[24 39 33 20]	[25 21 24 17]
Yersinia pestis	CO-92 Biovar Orientalis	NO DATA	[26 34 35 21]	[23 23 19 22]
Yersinia pestis	KIM5 P12 (Biovar Mediaevalis)	NO DATA	[26 34 35 21]	[23 23 19 22]
Yersinia pestis	91001	NO DATA	[26 34 35 21]	[23 23 19 22]
Haemophilus influenzae	KW20	NO DATA	NO DATA	NO DATA
Pseudomonas aeruginosa	PAO1	NO DATA	NO DATA	NO DATA
Pseudomonas fluorescens	Pf0-1	NO DATA	NO DATA	NO DATA
Pseudomonas putida	KT2440	NO DATA	[21 37 37 21]	NO DATA
Legionella pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella tularensis	schu 4	NO DATA	NO DATA	NO DATA
Bordetella pertussis	Tohama I	NO DATA	NO DATA	NO DATA
Burkholderia cepacia	J2315	NO DATA	NO DATA	NO DATA
Burkholderia pseudomallei	K96243	NO DATA	NO DATA	NO DATA
Neisseria gonorrhoeae	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	AR39	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium diphtheriae	NCTC13129	NO DATA	NO DATA	NO DATA
Mycobacterium avium	k10	NO DATA	NO DATA	NO DATA
Mycobacterium avium	104	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	CSU#93	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	CDC 1551	NO DATA	NO DATA	NO DATA
Mycobacterium tuberculosis	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
Mycoplasma pneumoniae	M129	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MRSA252	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MSSA476	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	COL	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	Mu50	NO DATA	NO DATA	NO DATA

Staphylococcus aureus	MW2	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	N315	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	NCTC 8325	NO DATA	NO DATA	NO DATA
Streptococcus agalactiae	NEM316	NO DATA	NO DATA	NO DATA
Streptococcus equi	NC_002955	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS8232	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS315	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	SSI-1	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS10394	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	Manfredo (M5)	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	SF370 (M1)	NO DATA	NO DATA	NO DATA
Streptococcus pneumoniae	670	NO DATA	NO DATA	NO DATA
Streptococcus pneumoniae	R6	NO DATA	NO DATA	NO DATA
Streptococcus pneumoniae	TIGR4	NO DATA	NO DATA	NO DATA
Streptococcus gordonii	NCTC7868	NO DATA	NO DATA	NO DATA
Streptococcus mitis	NCTC 12261	NO DATA	NO DATA	NO DATA
Streptococcus mutans	UA159	NO DATA	NO DATA	NO DATA

Table 7E – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 362, 363, and 367

Organism	Strain	Primer 362 [A G C T]	Primer 363 [A G C T]	Primer 367 [A G C T]
Klebsiella pneumoniae	MGH78578	[21 33 22 16]	[16 34 26 26]	NO DATA
Yersinia pestis	CO-92 Biovar Orientalis	[20 34 18 20]	NO DATA	NO DATA
Yersinia pestis	KIM5 P12 (Biovar Mediaevalis)	[20 34 18 20]	NO DATA	NO DATA
Yersinia pestis	91001	[20 34 18 20]	NO DATA	NO DATA
Haemophilus influenzae	KW20	NO DATA	NO DATA	NO DATA
Pseudomonas aeruginosa	PAO1	[19 35 21 17]	[16 36 28 22]	NO DATA
Pseudomonas fluorescens	Pf0-1	NO DATA	[18 35 26 23]	NO DATA
Pseudomonas putida	KT2440	NO DATA	[16 35 28 23]	NO DATA
Legionella pneumophila	Philadelphia-1	NO DATA	NO DATA	NO DATA
Francisella tularensis	schu 4	NO DATA	NO DATA	NO DATA
Bordetella pertussis	Tohama I	[20 31 24 17]	[15 34 32 21]	[26 25 34 19]
Burkholderia cepacia	J2315	[20 33 21 18]	[15 36 26 25]	[25 27 32 20]
Burkholderia pseudomallei	K96243	[19 34 19 20]	[15 37 28 22]	[25 27 32 20]
Neisseria gonorrhoeae	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA

Neisseria meningitidis	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
Neisseria meningitidis	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	TW-183	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	AR39	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	CWL029	NO DATA	NO DATA	NO DATA
Chlamydophila pneumoniae	J138	NO DATA	NO DATA	NO DATA
Corynebacterium diphtheriae	NCTC13129	NO DATA	NO DATA	NO DATA
Mycobacterium avium	k10	[19 34 23 16]	NO DATA	[24 26 35 19]
Mycobacterium avium	104	[19 34 23 16]	NO DATA	[24 26 35 19]
Mycobacterium tuberculosis	CSU#93	[19 31 25 17]	NO DATA	[25 25 34 20]
Mycobacterium tuberculosis	CDC 1551	[19 31 24 18]	NO DATA	[25 25 34 20]
Mycobacterium tuberculosis	H37Rv (lab strain)	[19 31 24 18]	NO DATA	[25 25 34 20]
Mycoplasma pneumoniae	M129	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MRSA252	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MSSA476	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	COL	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	Mu50	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	MW2	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	N315	NO DATA	NO DATA	NO DATA
Staphylococcus aureus	NCTC 8325	NO DATA	NO DATA	NO DATA
Streptococcus agalactiae	NEM316	NO DATA	NO DATA	NO DATA
Streptococcus equi	NC_002955	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS8232	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS315	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	SSI-1	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	MGAS10394	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	Manfredo (M5)	NO DATA	NO DATA	NO DATA
Streptococcus pyogenes	SF370 (M1)	NO DATA	NO DATA	NO DATA
Streptococcus pneumoniae	670	NO DATA	NO DATA	NO DATA
Streptococcus pneumoniae	R6	[20 30 19 23]	NO DATA	NO DATA
Streptococcus pneumoniae	TIGR4	[20 30 19 23]	NO DATA	NO DATA
Streptococcus gordonii	NCTC7868	NO DATA	NO DATA	NO DATA
Streptococcus mitis	NCTC 12261	NO DATA	NO DATA	NO DATA
Streptococcus	UA159	NO DATA	NO DATA	NO DATA

mutans				

[396] Four sets of throat samples from military recruits at different military facilities taken at different time points were analyzed using the primers of the present invention. The first set was collected at a military training center from November 1 to December 20, 2002 during one of the most severe outbreaks of pneumonia associated with group A *Streptococcus* in the United States since 1968. During this outbreak, fifty-one throat swabs were taken from both healthy and hospitalized recruits and plated on blood agar for selection of putative group A *Streptococcus* colonies. A second set of 15 original patient specimens was taken during the height of this group A *Streptococcus* -associated respiratory disease outbreak. The third set were historical samples, including twenty-seven isolates of group A *Streptococcus*, from disease outbreaks at this.and other military training facilities during previous years. The fourth set of samples was collected from five geographically separated military facilities in the continental U.S. in the winter immediately following the severe November/December 2002 outbreak.

[397] Pure colonies isolated from group A *Streptococcus*-selective media from all four collection periods were analyzed with the surveillance primer set. All samples showed base compositions that precisely matched the four completely sequenced strains of *Streptococcus pyogenes*. Shown in Figure 4 is a 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[398] In addition to the identification of *Streptococcus pyogenes*, other potentially pathogenic organisms were identified concurrently. Mass spectral analysis of a sample whose nucleic acid was amplified by primer pair number 349 (SEQ ID NOs: 401:1156) exhibited signals of bioagent identifying amplicons with molecular masses that were found to correspond to analogous base compositions of bioagent identifying amplicons of *Streptococcus pyogenes* (A27 G32 C24 T18), *Neisseria meningitidis* (A25 G27 C22 T18), and *Haemophilus influenzae* (A28 G28 C25 T20) (see Figure 5 and Table 7B). These organisms were present in a ratio of 4:5:20 as determined by comparison of peak heights with peak height of an internal PCR calibration standard as described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[399] Since certain division-wide primers that target housekeeping genes are designed to provide coverage of specific divisions of bacteria to increase the confidence level for identification of bacterial species, they are not expected to yield bioagent identifying amplicons for organisms outside of the specific divisions. For example, primer pair number 356 (SEQ ID NOs: 449:1380) primarily amplifies the nucleic acid of members of the classes *Bacilli* and *Clostridia* and is not expected to amplify proteobacteria such as *Neisseria meningitidis* and *Haemophilus influenzae*. As expected, analysis of the mass spectrum of amplification products obtained with primer pair number 356 does not indicate the presence of *Neisseria meningitidis* and *Haemophilus influenzae* but does indicate the presence of *Streptococcus pyogenes* (Figures 3 and 6, Table 7B). Thus, these primers or types of primers can confirm the absence of particular bioagents from a sample.

[400] The 15 throat swabs from military recruits were found to contain a relatively small set of microbes in high abundance. The most common were *Haemophilus influenza*, *Neisseria meningitides*, and *Streptococcus pyogenes*. *Staphylococcus epidermidis*, *Moraxella cattarhalis*, *Corynebacterium pseudodiphtheriticum*, and *Staphylococcus aureus* were present in fewer samples. An equal number of samples from healthy volunteers from three different geographic locations, were identically analyzed. Results indicated that the healthy volunteers have bacterial flora dominated by multiple, commensal non-beta-hemolytic *Streptococcal* species, including the viridans group *streptococci* (*S. parasangunis*, *S. vestibularis*, *S. mitis*, *S. oralis* and *S. pneumoniae*; data not shown), and none of the organisms found in the military recruits were found in the healthy controls at concentrations detectable by mass spectrometry. Thus, the military recruits in the midst of a respiratory disease outbreak had a dramatically different microbial population than that experienced by the general population in the absence of epidemic disease.

Example 7: Triangulation Genotyping Analysis for Determination of emm-Type of *Streptococcus pyogenes* in Epidemic Surveillance

[401] As a continuation of the epidemic surveillance investigation of Example 6, determination of sub-species characteristics (genotyping) of *Streptococcus pyogenes*, was carried out based on a strategy that generates strain-specific signatures according to the rationale of Multi-Locus Sequence Typing (MLST). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced (Enright et al. Infection and Immunity, 2001, 69, 2416-2427). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced. In the present investigation, bioagent identifying amplicons from housekeeping genes were produced using drill-down primers and analyzed by mass spectrometry. Since mass spectral analysis results in molecular mass,

from which base composition can be determined, the challenge was to determine whether resolution of *emm* classification of strains of *Streptococcus pyogenes* could be determined.

[402] For the purpose of development of a triangulation genotyping assay, an alignment was constructed of concatenated alleles of seven MLST housekeeping genes (glucose kinase (gki), glutamine transporter protein (gtr), glutamate racemase (murI), DNA mismatch repair protein (mutS), xanthine phosphoribosyl transferase (xpt), and acetyl-CoA acetyl transferase (yqiL)) from each of the 212 previously *emm*-typed strains of *Streptococcus pyogenes*. From this alignment, the number and location of primer pairs that would maximize strain identification via base composition was determined. As a result, 6 primer pairs were chosen as standard drill-down primers for determination of *emm*-type of *Streptococcus pyogenes*. These six primer pairs are displayed in Table 8. This drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

Table 8: Triangulation Genotyping Analysis Primer Pairs for Group A *Streptococcus* Drill-Down

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
442	SP101_SPET11_358_387_TMOD_F	588	SP101_SPET11_448_473_TMOD_R	998	gki
80	SP101_SPET11_358_387_F	126	SP101_SPET11_448_473_TMOD_R	766	gki
443	SP101_SPET11_600_629_TMOD_F	348	SP101_SPET11_686_714_TMOD_R	1018	gtr
81	SP101_SPET11_600_629_F	62	SP101_SPET11_686_714_R	772	gtr
426	SP101_SPET11_1314_1336_TMOD_F	363	SP101_SPET11_1403_1431_TMOD_R	849	murI
86	SP101_SPET11_1314_1336_F	68	SP101_SPET11_1403_1431_R	711	murI
430	SP101_SPET11_1807_1835_TMOD_F	235	SP101_SPET11_1901_1927_TMOD_R	1439	mutS
90	SP101_SPET11_1807_1835_F	33	SP101_SPET11_1901_1927_R	1412	mutS
438	SP101_SPET11_3075_3103_TMOD_F	473	SP101_SPET11_3168_3196_TMOD_R	875	xpt
96	SP101_SPET11_3075_3103_F	108	SP101_SPET11_3168_3196_R	715	xpt
441	SP101_SPET11_3511_3535_TMOD_F	531	SP101_SPET11_3605_3629_TMOD_R	1294	yqiL
98	SP101_SPET11_3511_3535_F	116	SP101_SPET11_3605_3629_R	832	yqiL

[403] The primers of Table 8 were used to produce bioagent identifying amplicons from nucleic acid present in the clinical samples. The bioagent identifying amplicons which were subsequently analyzed by mass spectrometry and base compositions corresponding to the molecular masses were calculated.

[404] Of the 51 samples taken during the peak of the November/December 2002 epidemic (Table 9A-C rows 1-3), all except three samples were found to represent *emm3*, a Group A *Streptococcus* genotype previously associated with high respiratory virulence. The three outliers were from samples obtained from healthy individuals and probably represent non-epidemic strains. Archived samples (Tables 9A-C rows 5-13) from historical collections showed a greater heterogeneity of base compositions and *emm* types as would be expected from different epidemics occurring at different places and dates. The results of the mass spectrometry analysis and *emm* gene sequencing were found to be concordant for the epidemic and historical samples.

Table 9A: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 426 and 430

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	murI (Primer Pair No. 426)	mutS (Primer Pair No. 430)
48	3	3	MCRD San Diego (Cultured)	2002	A39 G25 C20 T34	A38 G27 C23 T33
2	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
15	3	ND			A39 G25 C20 T34	A38 G27 C23 T33
6	3	3	NHRC San Diego-Archive (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
3	5,58	5			A40 G24 C20 T34	A38 G27 C23 T33
6	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
3	12	12			A40 G24 C20 T34	A38 G26 C24 T33
1	22	22			A39 G25 C20 T34	A38 G27 C23 T33
3	25,75	75			A39 G25 C20 T34	A38 G27 C23 T33
4	44/61,82,9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	53,91	91			A39 G25 C20 T34	A38 G27 C23 T33
1	2	2	Ft. Leonard Wood (Cultured)	2003	A39 G25 C20 T34	A38 G27 C24 T32
2	3	3			A39 G25 C20 T34	A38 G27 C23 T33
1	4	4			A39 G25 C20 T34	A38 G27 C23 T33
1	6	6			A40 G24 C20 T34	A38 G27 C23 T33
11	25 or 75	75			A39 G25 C20 T34	A38 G27 C23 T33
1	25,75, 33, 34,4,52,84	75			A39 G25 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	5 or 58	5			A40 G24 C20 T34	A38 G27 C23 T33
3	1	1	Ft. Sill (Cultured)	2003	A40 G24 C20 T34	A38 G27 C23 T33
2	3	3			A39 G25 C20 T34	A38 G27 C23 T33
1	4	4			A39 G25 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
1	3	3	Ft.	2003	A39 G25 C20 T34	A38 G27 C23 T33

1	4	4	Benning (Cultured)		A39 G25 C20 T34	A38 G27 C23 T33
3	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
1	13	94**			A40 G24 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	82			A40 G24 C20 T34	A38 G26 C24 T33
1	5 or 58	58			A40 G24 C20 T34	A38 G27 C23 T33
1	78 or 89	89			A39 G25 C20 T34	A38 G27 C23 T33
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A40 G24 C20 T34	A38 G27 C23 T33
1	2				A39 G25 C20 T34	A38 G27 C24 T32
1	81 or 90				A40 G24 C20 T34	A38 G27 C23 T33
1	78				A38 G26 C20 T34	A38 G27 C23 T33
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A39 G25 C20 T34	A38 G27 C23 T33
1	3	ND			No detection	A38 G27 C23 T33
1	3	ND			No detection	No detection
1	3	ND			No detection	No detection
2	3	ND			No detection	A38 G27 C23 T33
3	No detection	ND			No detection	No detection

Table 9B: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	xpt (Primer Pair No. 438)	yqiL (Primer Pair No. 441)
48	3	3	MCRD San Diego (Cultured)	2002	A30 G36 C20 T36	A40 G29 C19 T31
2	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	28	28			A30 G36 C20 T36	A41 G28 C18 T32
15	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
6	3	3	NHRC San Diego-Archive (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
3	5,58	5			A30 G36 C20 T36	A40 G29 C19 T31
6	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
3	12	12			A30 G36 C19 T37	A40 G29 C19 T31
1	22	22			A30 G36 C20 T36	A40 G29 C19 T31
3	25,75	75			A30 G36 C20 T36	A40 G29 C19 T31
4	44/61,82,9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	53,91	91			A30 G36 C19 T37	A40 G29 C19 T31
1	2	2	Ft. Leonard Wood (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
2	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	6	6			A30 G36 C20 T36	A40 G29 C19 T31
11	25 or 75	75			A30 G36 C20 T36	A40 G29 C19 T31
1	25,75, 33, 34,4,52,84	75			A30 G36 C19 T37	A40 G29 C19 T31
1	44/61 or 82 or 9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	5			A30 G36 C20 T36	A40 G29 C19 T31
3	1	1	Ft. Sill (Cultured)	2003	A30 G36 C19 T37	A40 G29 C19 T31
2	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31

1	28	28			A30 G36 C20 T36	A41 G28 C18 T32
1	3	3	Ft. Benning (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
3	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
1	13	94**			A30 G36 C20 T36	A41 G28 C19 T31
1	44/61 or 82 or 9	82			A30 G36 C20 T36	A41 G28 C19 T31
1	5 or 58	58			A30 G36 C20 T36	A40 G29 C19 T31
1	78 or 89	89			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	2				A30 G36 C20 T36	A40 G29 C19 T31
1	81 or 90				A30 G36 C20 T36	A40 G29 C19 T31
1	78				A30 G36 C20 T36	A41 G28 C19 T31
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	No detection
1	3	ND			No detection	A40 G29 C19 T31
2	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
3	No detection	ND			No detection	No detection

Table 9C: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	gki (Primer Pair No. 442)	gtr ((Primer Pair No. 443)
48	3	3	MCRD San Diego (Cultured)	2002	A32 G35 C17 T32	A39 G28 C16 T32
2	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
15	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
6	3	3	NHRC San Diego-Archive (Cultured)	2003	A32 G35 C17 T32	A39 G28 C16 T32
3	5,58	5			A30 G36 C20 T30	A39 G28 C15 T33
6	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
3	12	12			A31 G35 C17 T33	A39 G28 C15 T33
1	22	22			A31 G35 C17 T33	A38 G29 C15 T33
3	25,75	75			A30 G36 C17 T33	A39 G28 C15 T33
4	44/61,82,9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	53,91	91			A32 G35 C17 T32	A39 G28 C16 T32
1	2	2	Ft. Leonard Wood (Cultured)	2003	A30 G36 C17 T33	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
1	6	6			A31 G35 C17 T33	A39 G28 C15 T33
11	25 or 75	75			A30 G36 C17 T33	A39 G28 C15 T33
1	25,75, 33, 34,4,52,84	75			A30 G36 C17 T33	A39 G28 C15 T33
1	44/61 or 82 or 9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	5			A30 G36 C20 T30	A39 G28 C15 T33
3	1	1	Ft. Sill	2003	A30 G36 C18 T32	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32

1	4	4	(Cultured)		A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
1	3	3	Ft. Benning (Cultured)	2003	A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
3	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
1	13	94**			A30 G36 C19 T31	A39 G28 C15 T33
1	44/61 or 82 or 9	82			A30 G36 C18 T32	A39 G28 C15 T33
1	5 or 58	58			A30 G36 C20 T30	A39 G28 C15 T33
1	78 or 89	89			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A30 G36 C20 T30	A39 G28 C15 T33
1	2				A30 G36 C17 T33	A39 G28 C15 T33
1	81 or 90				A30 G36 C17 T33	A39 G28 C15 T33
1	78				A30 G36 C18 T32	A39 G28 C15 T33
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			No detection	No detection
1	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			A32 G35 C17 T32	No detection
2	3	ND			A32 G35 C17 T32	No detection
3	No detection	ND			No detection	No detection

Example 8: Design of Calibrant Polynucleotides based on Bioagent Identifying Amplicons for Identification of Species of Bacteria (Bacterial Bioagent Identifying Amplicons)

[405] This example describes the design of 19 calibrant polynucleotides based on bacterial bioagent identifying amplicons corresponding to the primers of the broad surveillance set (Table 5) and the *Bacillus anthracis* drill-down set (Table 6).

[406] Calibration sequences were designed to simulate bacterial bioagent identifying amplicons produced by the T modified primer pairs shown in Tables 5 and 6 (primer names have the designation "TMOD"). The calibration sequences were chosen as a representative member of the section of bacterial genome from specific bacterial species which would be amplified by a given primer pair. The model bacterial species upon which the calibration sequences are based are also shown in Table 10. For example, the calibration sequence chosen to correspond to an amplicon produced by primer pair no. 361 is SEQ ID NO: 1445. In Table 10, the forward (_F) or reverse (_R) primer name indicates the coordinates of an extraction representing a gene of a standard reference bacterial genome to which the primer hybridizes e.g.: the forward primer name 16S_EC_713_732_TMOD_F indicates that the forward primer hybridizes to residues 713-732 of the gene encoding 16S ribosomal RNA in an *E. coli* reference sequence (in this case, the reference sequence is an extraction consisting of residues 4033120-4034661 of the genomic sequence of *E. coli* K12 (GenBank gi number 16127994). Additional gene coordinate reference information is shown in Table 11. The designation "TMOD" in the primer names indicates that the 5' end of the primer has been modified with a non-matched template T residue which

prevents the PCR polymerase from adding non-templated adenosine residues to the 5' end of the amplification product, an occurrence which may result in miscalculation of base composition from molecular mass data (*vide supra*).

[0143] The 19 calibration sequences described in Tables 10 and 11 were combined into a single calibration polynucleotide sequence (SEQ ID NO: 1464 - which is herein designated a "combination calibration polynucleotide") which was then cloned into a pCR®-Blunt vector (Invitrogen, Carlsbad, CA). This combination calibration polynucleotide can be used in conjunction with the primers of Tables 5 or 6 as an internal standard to produce calibration amplicons for use in determination of the quantity of any bacterial bioagent. Thus, for example, when the combination calibration polynucleotide vector is present in an amplification reaction mixture, a calibration amplicon based on primer pair 346 (16S rRNA) will be produced in an amplification reaction with primer pair 346 and a calibration amplicon based on primer pair 363 (rpoC) will be produced with primer pair 363. Coordinates of each of the 19 calibration sequences within the calibration polynucleotide (SEQ ID NO: 1464) are indicated in Table 11.

Table 10: Bacterial Primer Pairs for Production of Bacterial Bioagent Identifying Amplicons and Corresponding Representative Calibration Sequences

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Calibration Sequence Model Species	Calibration Sequence (SEQ ID NO:)
361	16S_EC_1090_1111_2_TMOD_F	697	16S_EC_1175_1196_TMOD_R	1398	*Bacillus anthracis*	1445
346	16S_EC_713_732_TMOD_F	202	16S_EC_789_809_TMOD_R	1110	*Bacillus anthracis*	1446
347	16S_EC_785_806_TMOD_F	560	16S_EC_880_897_TMOD_R	1278	*Bacillus anthracis*	1447
348	16S_EC_960_981_TMOD_F	706	16S_EC_1054_1073_TMOD_R	895	*Bacillus anthracis*	1448
349	23S_EC_1826_1843_TMOD_F	401	23S_EC_1906_1924_TMOD_R	1156	*Bacillus anthracis*	1449
360	23S_EC_2646_2667_TMOD_F	409	23S_EC_2745_2765_TMOD_R	1434	*Bacillus anthracis*	1450
350	CAPC_BA_274_303_TMOD_F	476	CAPC_BA_349_376_TMOD_R	1314	*Bacillus anthracis*	1451
351	CYA_BA_1353_1379_TMOD_F	355	CYA_BA_1448_1467_TMOD_R	1423	*Bacillus anthracis*	1452
352	INFB_EC_1365_1393_TMOD_F	687	INFB_EC_1439_1467_TMOD_R	1411	*Bacillus anthracis*	1453
353	LEF_BA_756_781_TMOD_F	220	LEF_BA_843_872_TMOD_R	1394	*Bacillus anthracis*	1454
356	RPLB_EC_650_679_TMOD_F	449	RPLB_EC_739_762_TMOD_R	1380	*Clostridium botulinum*	1455
449	RPLB_EC_690_710_F	309	RPLB_EC_737_758_R	1336	*Clostridium botulinum*	1456
359	RPOB_EC_1845_1866_TMOD_F	659	RPOB_EC_1909_1929_TMOD_R	1250	*Yersinia Pestis*	1457
362	RPOB_EC_3799_3821_TMOD_F	581	RPOB_EC_3862_3888_TMOD_R	1325	*Burkholderia mallei*	1458
363	RPOC_EC_2146_2174_TMOD_F	284	RPOC_EC_2227_2245_TMOD_R	898	*Burkholderia mallei*	1459

354	RPOC_EC_2218_2241_TMOD_F	405	RPOC_EC_2313_2337_TMOD_R	1072	*Bacillus anthracis*	1460
355	SSPE_BA_115_137_TMOD_F	255	SSPE_BA_197_222_TMOD_R	1402	*Bacillus anthracis*	1461
367	TUFB_EC_957_979_TMOD_F	308	TUFB_EC_1034_1058_TMOD_R	1276	*Burkholderia mallei*	1462
358	VALS_EC_1105_1124_TMOD_F	385	VALS_EC_1195_1218_TMOD_R	1093	*Yersinia Pestis*	1463

Table 11: Primer Pair Gene Coordinate References and Calibration Polynucleotide Sequence Coordinates within the Combination Calibration Polynucleotide

Bacterial Gene and Species	Gene Extraction Coordinates of Genomic or Plasmid Sequence	Reference GenBank GI No. of Genomic (G) or Plasmid (P) Sequence	Primer Pair No.	Coordinates of Calibration Sequence in Combination Calibration Polynucleotide (SEQ ID NO: 1464)
16S *E. coli*	4033120..4034661	16127994 (G)	346	16..109
16S *E. coli*	4033120..4034661	16127994 (G)	347	83..190
16S *E. coli*	4033120..4034661	16127994 (G)	348	246..353
16S *E. coli*	4033120..4034661	16127994 (G)	361	368..469
23S *E. coli*	4166220..4169123	16127994 (G)	349	743..837
23S *E. coli*	4166220..4169123	16127994 (G)	360	865..981
rpoB *E. coli.*	4178823..4182851 (complement strand)	16127994 (G)	359	1591..1672
rpoB *E. coli*	4178823..4182851 (complement strand)	16127994 (G)	362	2081..2167
rpoC *E. coli*	4182928..4187151	16127994 (G)	354	1810..1926
rpoC *E. coli*	4182928..4187151	16127994 (G)	363	2183..2279
infB *E. coli*	3313655..3310983 (complement strand)	16127994 (G)	352	1692..1791
tufB *E. coli*	4173523..4174707	16127994 (G)	367	2400..2498
rplB *E. coli*	3449001..3448180	16127994 (G)	356	1945..2060
rplB *E. coli*	3449001..3448180	16127994 (G)	449	1986..2055
valS *E. coli*	4481405..4478550 (complement strand)	16127994 (G)	358	1462..1572
capC *B. anthracis*	56074..55628 (complement strand)	6470151 (P)	350	2517..2616
cya *B. anthracis*	156626..154288 (complement strand)	4894216 (P)	351	1338..1449
lef *B. anthracis*	127442..129921	4894216 (P)	353	1121..1234
sspE *B. anthracis*	226496..226783	30253828 (G)	355	1007-1104

Example 9: Use of a Calibration Polynucleotide for Determining the Quantity of *Bacillus Anthracis* in a Sample Containing a Mixture of Microbes

[407] The process described in this example is shown in Figure 2. The capC gene is a gene involved in capsule synthesis which resides on the pX02 plasmid of *Bacillus anthracis*. Primer pair number 350 (see Tables 10 and 11) was designed to identify *Bacillus anthracis* via production of a bacterial bioagent identifying amplicon. Known quantities of the combination calibration polynucleotide vector described in Example 8 were added to amplification mixtures containing bacterial bioagent nucleic acid from a mixture of microbes which included the Ames strain of *Bacillus anthracis*. Upon amplification of the bacterial bioagent nucleic acid and the combination calibration polynucleotide vector with primer pair no. 350, bacterial bioagent identifying amplicons and calibration amplicons were obtained and characterized by mass spectrometry. A mass spectrum measured for the amplification reaction is shown in Figure 7. The molecular masses of the bioagent identifying amplicons provided the means for identification of the bioagent from which they were obtained (Ames strain of *Bacillus*

anthracis) and the molecular masses of the calibration amplicons provided the means for their identification as well. The relationship between the abundance (peak height) of the calibration amplicon signals and the bacterial bioagent identifying amplicon signals provides the means of calculation of the copies of the pX02 plasmid of the Ames strain of *Bacillus anthracis*. Methods of calculating quantities of molecules based on internal calibration procedures are well known to those of ordinary skill in the art.

[408] Averaging the results of 10 repetitions of the experiment described above, enabled a calculation that indicated that the quantity of Ames strain of *Bacillus anthracis* present in the sample corresponds to approximately 10 copies of pX02 plasmid.

Example 10: Triangulation Genotyping Analysis of *Campylobacter* Species

[409] A series of triangulation genotyping analysis primers were designed as described in Example 1 with the objective of identification of different strains of *Campylobacter jejuni*. The primers are listed in Table 12 with the designation "CJST_CJ." Housekeeping genes to which the primers hybridize and produce bioagent identifying amplicons include: tkt (transketolase), glyA (serine hydroxymethyltransferase), gltA (citrate synthase), aspA (aspartate ammonia lyase), glnA (glutamine synthase), pgm (phosphoglycerate mutase), and uncA (ATP synthetase alpha chain).

Table 12: *Campylobacter* Genotyping Primer Pairs

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1053	CJST_CJ_1080_1110_F	681	CJST_CJ_1166_1198_R	1022	gltA
1047	CJST_CJ_584_616_F	315	CJST_CJ_663_692_R	1379	glnA
1048	CJST_CJ_360_394_F	346	CJST_CJ_442_476_R	955	aspA
1049	CJST_CJ_2636_2668_F	504	CJST_CJ_2753_2777_R	1409	tkt
1054	CJST_CJ_2060_2090_F	323	CJST_CJ_2148_2174_R	1068	pgm
1064	CJST_CJ_1680_1713_F	479	CJST_CJ_1795_1822_R	938	glyA

[410] The primers were used to amplify nucleic acid from 50 food product samples provided by the USDA, 25 of which contained *Campylobacter jejuni* and 25 of which contained *Campylobacter coli*. Primers used in this study were developed primarily for the discrimination of *Campylobacter jejuni* clonal complexes and for distinguishing *Campylobacter jejuni* from *Campylobacter coli*. Finer discrimination between *Campylobacter coli* types is also possible by using specific primers targeted to loci where closely-related *Campylobacter coli* isolates demonstrate polymorphisms between strains. The conclusions of the comparison of base composition analysis with sequence analysis are shown in Tables 13A-C.

Table 13A – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1048 and 1047

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1048 (aspA)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1047 (glnA)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A30 G25 C16 T46	A47 G21 C16 T25
J-2	C. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A30 G25 C16 T46	A48 G21 C17 T23
J-3	C. jejuni	Human	Complex 354/179	ST 436	RM4194	A30 G25 C15 T47	A48 G21 C18 T22
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A30 G25 C16 T46	A48 G21 C18 T22
J-5	C. jejuni	Human	Complex 52	ST 52, complex 52	RM4277	A30 G25 C16 T46	A48 G21 C17 T23
J-6	C. jejuni	Human	Complex 443	ST 51, complex 443	RM4275	A30 G25 C15 T47	A48 G21 C17 T23
					RM4279	A30 G25 C15 T47	A48 G21 C17 T23
J-7	C. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A30 G25 C15 T47	A48 G21 C18 T22
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A30 G25 C15 T47	A48 G21 C18 T22
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A30 G25 C15 T47	A47 G21 C18 T23
	C. jejuni	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A31 G27 C20 T39	A48 G21 C16 T24
C-1	C. coli			ST 832	RM1169	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1056	RM1857	A31 G27 C20 T39	A48 G21 C16 T24
		Poultry		ST 889	RM1166	A31 G27 C20 T39	A48 G21 C16 T24
				ST 829	RM1182	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1050	RM1518	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1051	RM1521	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1053	RM1523	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1055	RM1527	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1017	RM1529	A31 G27 C20 T39	A48 G21 C16 T24
				ST 860	RM1840	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1063	RM2219	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1066	RM2241	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1067	RM2243	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1068	RM2439	A31 G27 C20 T39	A48 G21 C16 T24
		Swine		ST 1016	RM3230	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1069	RM3231	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1061	RM1904	A31 G27 C20 T39	A48 G21 C16 T24
		Unknown		ST 825	RM1534	A31 G27 C20 T39	A48 G21 C16 T24
				ST 901	RM1505	A31 G27 C20 T39	A48 G21 C16 T24
C-2	C. coli	Human	ST 895	ST 895	RM1532	A31 G27 C19 T40	A48 G21 C16 T24
C-3	C. coli	Poultry	Consistent	ST 1064	RM2223	A31 G27 C20 T39	A48 G21 C16 T24

			with 63 closely related sequence types (none belong to a clonal complex)	ST 1082	RM1178	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1054	RM1525	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1049	RM1517	A31 G27 C20 T39	A48 G21 C16 T24
		Marmoset		ST 891	RM1531	A31 G27 C20 T39	A48 G21 C16 T24

Table 13B – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1053 and 1064

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1053 (gltA)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1064 (glyA)
J-1	*C. jejuni*	Goose	ST 690 /692/707/991	ST 991	RM3673	A24 G25 C23 T47	A40 G29 C29 T45
J-2	*C. jejuni*	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A24 G25 C23 T47	A40 G29 C29 T45
J-3	*C. jejuni*	Human	Complex 354/179	ST 436	RM4194	A24 G25 C23 T47	A40 G29 C29 T45
J-4	*C. jejuni*	Human	Complex 257	ST 257, complex 257	RM4197	A24 G25 C23 T47	A40 G29 C29 T45
J-5	*C. jejuni*	Human	Complex 52	ST 52, complex 52	RM4277	A24 G25 C23 T47	A39 G30 C26 T48
J-6	*C. jejuni*	Human	Complex 443	ST 51, complex 443	RM4275	A24 G25 C23 T47	A39 G30 C28 T46
					RM4279	A24 G25 C23 T47	A39 G30 C28 T46
J-7	*C. jejuni*	Human	Complex 42	ST 604, complex 42	RM1864	A24 G25 C23 T47	A39 G30 C26 T48
J-8	*C. jejuni*	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A24 G25 C23 T47	A38 G31 C28 T46
J-9	*C. jejuni*	Human	Complex 45/283	ST 147, Complex 45	RM3203	A24 G25 C23 T47	A38 G31 C28 T46
	C. jejuni	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A23 G24 C26 T46	A39 G30 C27 T47
C-1	*C. coli*			ST 832	RM1169	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1056	RM1857	A23 G24 C26 T46	A39 G30 C27 T47
		Poultry		ST 889	RM1166	A23 G24 C26 T46	A39 G30 C27 T47
				ST 829	RM1182	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1050	RM1518	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1051	RM1521	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1053	RM1523	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1055	RM1527	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1017	RM1529	A23 G24 C26 T46	A39 G30 C27 T47
				ST 860	RM1840	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1063	RM2219	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1066	RM2241	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1067	RM2243	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1068	RM2439	A23 G24 C26 T46	A39 G30 C27 T47
		Swine		ST 1016	RM3230	A23 G24 C26 T46	A39 G30 C27 T47

				ST 1069	RM3231	A23 G24 C26 T46	NO DATA
				ST 1061	RM1904	A23 G24 C26 T46	A39 G30 C27 T47
		Unknown		ST 825	RM1534	A23 G24 C26 T46	A39 G30 C27 T47
				ST 901	RM1505	A23 G24 C26 T46	A39 G30 C27 T47
C-2	C. coli	Human	ST 895	ST 895	RM1532	A23 G24 C26 T46	A39 G30 C27 T47
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1082	RM1178	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1054	RM1525	A23 G24 C25 T47	A39 G30 C27 T47
				ST 1049	RM1517	A23 G24 C26 T46	A39 G30 C27 T47
		Marmoset		ST 891	RM1531	A23 G24 C26 T46	A39 G30 C27 T47

Table 13C – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1054 and 1049

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1054 (pgm)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1049 (tkt)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A26 G33 C18 T38	A41 G28 C35 T38
J-2	C. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A26 G33 C19 T37	A41 G28 C36 T37
J-3	C. jejuni	Human	Complex 354/179	ST 436	RM4194	A27 G32 C19 T37	A42 G28 C36 T36
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A27 G32 C19 T37	A41 G29 C35 T37
J-5	C. jejuni	Human	Complex 52	ST 52, complex 52	RM4277	A26 G33 C18 T38	A41 G28 C36 T37
J-6	C. jejuni	Human	Complex 443	ST 51, complex 443	RM4275	A27 G31 C19 T38	A41 G28 C36 T37
					RM4279	A27 G31 C19 T38	A41 G28 C36 T37
J-7	C. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A27 G32 C19 T37	A42 G28 C35 T37
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A26 G33 C19 T37	A42 G28 C35 T37
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A28 G31 C19 T37	A43 G28 C36 T35
	C. jejuni	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A27 G30 C19 T39	A46 G28 C32 T36
C-1	C. coli			ST 832	RM1169	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1056	RM1857	A27 G30 C19 T39	A46 G28 C32 T36
		Poultry		ST 889	RM1166	A27 G30 C19 T39	A46 G28 C32 T36
				ST 829	RM1182	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1050	RM1518	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1051	RM1521	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1053	RM1523	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1055	RM1527	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1017	RM1529	A27 G30 C19 T39	A46 G28 C32 T36

				ST 860	RM1840	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1063	RM2219	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1066	RM2241	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1067	RM2243	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1068	RM2439	A27 G30 C19 T39	A46 G28 C32 T36
		Swine		ST 1016	RM3230	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1069	RM3231	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1061	RM1904	A27 G30 C19 T39	A46 G28 C32 T36
		Unknown		ST 825	RM1534	A27 G30 C19 T39	A46 G28 C32 T36
				ST 901	RM1505	A27 G30 C19 T39	A46 G28 C32 T36
C-2	*C. coli*	Human	ST 895	ST 895	RM1532	A27 G30 C19 T39	A45 G29 C32 T36
C-3	*C. coli*	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1082	RM1178	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1054	RM1525	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1049	RM1517	A27 G30 C19 T39	A45 G29 C32 T36
		Marmoset		ST 891	RM1531	A27 G30 C19 T39	A45 G29 C32 T36

[411] The base composition analysis method was successful in identification of 12 different strain groups. *Campylobacter jejuni* and *Campylobacter coli* are generally differentiated by all loci. Ten clearly differentiated *Campylobacter jejuni* isolates and 2 major *Campylobacter coli* groups were identified even though the primers were designed for strain typing of *Campylobacter jejuni*. One isolate (RM4183) which was designated as *Campylobacter jejuni* was found to group with *Campylobacter coli* and also appears to actually be *Campylobacter coli* by full MLST sequencing.

Example 11: Identification of *Acinetobacter baumannii* Using Broad Range Survey and Division-Wide Primers in Epidemiological Surveillance

[412] To test the capability of the broad range survey and division-wide primer sets of Table 5 in identification of *Acinetobacter* species, 183 clinical samples were obtained from individuals participating in, or in contact with individuals participating in Operation Iraqi Freedom (including US service personnel, US civilian patients at the Walter Reed Army Institute of Research (WRAIR), medical staff, Iraqi civilians and enemy prisoners. In addition, 34 environmental samples were obtained from hospitals in Iraq, Kuwait, Germany, the United States and the USNS Comfort, a hospital ship.

[413] Upon amplification of nucleic acid obtained from the clinical samples, primer pairs 346-349, 360, 361, 354, 362 and 363 (Table 5) all produced bacterial bioagent amplicons which identified *Acinetobacter baumannii* in 215 of 217 samples. The organism *Klebsiella pneumoniae* was identified in the remaining two samples. In addition, 14 different strain types (containing single nucleotide polymorphisms relative to a reference strain of *Acinetobacter baumannii*) were identified and assigned

arbitrary numbers from 1 to 14. Strain type 1 was found in 134 of the sample isolates and strains 3 and 7 were found in 46 and 9 of the isolates respectively.

[414] The epidemiology of strain type 7 of *Acinetobacter baumannii* was investigated. Strain 7 was found in 4 patients and 5 environmental samples (from field hospitals in Iraq and Kuwait). The index patient infected with strain 7 was a pre-war patient who had a traumatic amputation in March of 2003 and was treated at a Kuwaiti hospital. The patient was subsequently transferred to a hospital in Germany and then to WRAIR. Two other patients from Kuwait infected with strain 7 were found to be non-infectious and were not further monitored. The fourth patient was diagnosed with a strain 7 infection in September of 2003 at WRAIR. Since the fourth patient was not related involved in Operation Iraqi Freedom, it was inferred that the fourth patient was the subject of a nosocomial infection acquired at WRAIR as a result of the spread of strain 7 from the index patient.

[415] The epidemiology of strain type 3 of *Acinetobacter baumannii* was also investigated. Strain type 3 was found in 46 samples, all of which were from patients (US service members, Iraqi civilians and enemy prisoners) who were treated on the USNS Comfort hospital ship and subsequently returned to Iraq or Kuwait. The occurrence of strain type 3 in a single locale may provide evidence that at least some of the infections at that locale were a result of nosocomial infections.

[416] This example thus illustrates an embodiment of the present invention wherein the methods of analysis of bacterial bioagent identifying amplicons provide the means for epidemiological surveillance.

Example 12: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for *Acinetobacter baumanii*

[417] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, an additional 21 primer pairs were selected based on analysis of housekeeping genes of the genus *Acinetobacter*. Genes to which the drill-down triangulation genotyping analysis primers hybridize for production of bacterial bioagent identifying amplicons include anthranilate synthase component I (trpE), adenylate kinase (adk), adenine glycosylase (mutY), fumarate hydratase (fumC), and pyrophosphate phospho-hydratase (ppa). These 21 primer pairs are indicated with reference to sequence listings in Table 14. Primer pair numbers 1151-1154 hybridize to and amplify segments of trpE. Primer pair numbers 1155-1157 hybridize to and amplify segments of adk. Primer pair numbers 1158-1164 hybridize to and amplify segments of mutY. Primer pair numbers 1165-1170 hybridize to and amplify segments of fumC. Primer pair number 1171 hybridizes to and amplifies a segment of ppa.

Primer pair numbers: 2846-2848 hybridize to and amplify segments of the parC gene of DNA topoisomerase which include a codon known to confer quinolone drug resistance upon sub-types of *Acinetobacter baumannii.* Primer pair numbers 2852-2854 hybridize to and amplify segments of the gyrA gene of DNA gyrase which include a codon known to confer quinolone drug resistance upon sub-types of *Acinetobacter baumannii.* Primer pair numbers 2922 and 2972 are speciating primers which are useful for identifying different species members of the genus *Acinetobacter*. The primer names given in Table 14A (with the exception of primer pair numbers 2846-2848, 2852-2854) indicate the coordinates to which the primers hybridize to a reference sequence which comprises a concatenation of the genes TrpE, efp (elongation factor p), adk, mutT, fumC, and ppa. For example, the forward primer of primer pair 1151 is named AB_MLST-11-OIF007_62_91_F because it hybridizes to the *Acinetobacter* primer reference sequence of strain type 11 in sample 007 of Operation Iraqi Freedom (OIF) at positions 62 to 91. DNA was sequenced from strain type 11 and from this sequence data and an artificial concatenated sequence of partial gene extractions was assembled for use in design of the triangulation genotyping analysis primers. The stretches of arbitrary residues "N"s in the concatenated sequence were added for the convenience of separation of the partial gene extractions (40N for AB_MLST (SEQ ID NO: 1444)).

[418] The hybridization coordinates of primer pair numbers 2846-2848 are with respect to GenBank Accession number X95819. The hybridization coordinates of primer pair numbers 2852-2854 are with respect to GenBank Accession number AY642140. Sequence residue "I" appearing in the forward and reverse primers of primer pair number 2972 represents inosine.

Table 14A: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus *Acinetobacter*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)
1151	AB_MLST-11-OIF007_62_91_F	454	AB_MLST-11-OIF007_169_203_R	1418
1152	AB_MLST-11-OIF007_185_214_F	243	AB_MLST-11-OIF007_291_324_R	969
1153	AB_MLST-11-OIF007_260_289_F	541	AB_MLST-11-OIF007_364_393_R	1400
1154	AB_MLST-11-OIF007_206_239_F	436	AB_MLST-11-OIF007_318_344_R	1036
1155	AB_MLST-11-OIF007_522_552_F	378	AB_MLST-11-OIF007_587_610_R	1392
1156	AB_MLST-11-OIF007_547_571_F	250	AB_MLST-11-OIF007_656_686_R	902
1157	AB_MLST-11-OIF007_601_627_F	256	AB_MLST-11-OIF007_710_736_R	881
1158	AB_MLST-11-OIF007_1202_1225_F	384	AB_MLST-11-OIF007_1266_1296_R	878
1159	AB_MLST-11-OIF007_1202_1225_F	384	AB_MLST-11-OIF007_1299_1316_R	1199
1160	AB_MLST-11-OIF007_1234_1264_F	694	AB_MLST-11-OIF007_1335_1362_R	1215

1161	AB_MLST-11-OIF007_1327_1356_F	225	AB_MLST-11-OIF007_1422_1448_R	1212
1162	AB_MLST-11-OIF007_1345_1369_F	383	AB_MLST-11-OIF007_1470_1494_R	1083
1163	AB_MLST-11-OIF007_1351_1375_F	662	AB_MLST-11-OIF007_1470_1494_R	1083
1164	AB_MLST-11-OIF007_1387_1412_F	422	AB_MLST-11-OIF007_1470_1494_R	1083
1165	AB_MLST-11-OIF007_1542_1569_F	194	AB_MLST-11-OIF007_1656_1680_R	1173
1166	AB_MLST-11-OIF007_1566_1593_F	684	AB_MLST-11-OIF007_1656_1680_R	1173
1167	AB_MLST-11-OIF007_1611_1638_F	375	AB_MLST-11-OIF007_1731_1757_R	890
1168	AB_MLST-11-OIF007_1726_1752_F	182	AB_MLST-11-OIF007_1790_1821_R	1195
1169	AB_MLST-11-OIF007_1792_1826_F	656	AB_MLST-11-OIF007_1876_1909_R	1151
1170	AB_MLST-11-OIF007_1792_1826_F	656	AB_MLST-11-OIF007_1895_1927_R	1224
1171	AB_MLST-11-OIF007_1970_2002_F	618	AB_MLST-11-OIF007_2097_2118_R	1157
2846	PARC_X95819_33_58_F	302	PARC_X95819_121_153_R	852
2847	PARC_X95819_33_58_F	199	PARC_X95819_157_178_R	889
2848	PARC_X95819_33_58_F	596	PARC_X95819_97_128_R	1169
2852	GYRA_AY642140_-1_24_F	150	GYRA_AY642140_71_100_R	1242
2853	GYRA_AY642140_26_54_F	166	GYRA_AY642140_121_146_R	1069
2854	GYRA_AY642140_26_54_F	166	GYRA_AY642140_58_89_R	1168
2922	AB_MLST-11-OIF007_991_1018_F	583	AB_MLST-11-OIF007_1110_1137_R	923
2972	AB_MLST-11-OIF007_1007_1034_F	592	AB_MLST-11-OIF007_1126_1153_R	924

Table 14B: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus *Acinetobacter*

Primer Pair No.	Forward Primer (SEQ ID NO:)	SEQUENCE	Reverse Primer (SEQ ID NO:)	SEQUENCE
1151	454	TGAGATTGCTGAACATTTAATGCTGATTGA	1418	TTGTACATTTGAAACAATATGCATGACATGTGAAT
1152	243	TATTGTTTCAAATGTACAAGGTGAAGTGCG	969	TCACAGGTTCTACTTCATCAATAATTTCCATTGC
1153	541	TGGAACGTTATCAGGTGCCCCAAAAATTCG	1400	TTGCAATCGACATATCCATTTCACCATGCC
1154	436	TGAAGTGCGTGATGATATCGATGCACTTGATGTA	1036	TCCGCCAAAAACTCCCCTTTTCACAGG
1155	378	TCGGTTTAGTAAAAGAACGTATTGCTCAACC	1392	TTCTGCTTGAGGAATAGTGCGTGG
1156	250	TCAACCTGACTGCGTGAATGGTTGT	902	TACGTTCTACGATTTCTTCATCAGGTACATC
1157	256	TCAAGCAGAAGCTTTGGAAGAAGAAGG	881	TACAACGTGATAAACACGACCAGAAGC
1158	384	TCGTGCCCGCAATTTGCATAAAGC	878	TAATGCCGGGTAGTGCAATCCATTCTTCTAG
1159	384	TCGTGCCCGCAATTTGCATAAAGC	1199	TGCACCTGCGGTCGAGCG
1160	694	TTGTAGCACAGCAAGGCAAATTTCCTGAAAC	1215	TGCCATCCATAATCACGCCATACTGACG
1161	225	TAGGTTTACGTCAGTATGGCGTGATTATGG	1212	TGCCAGTTTCCACATTTCACGTTCGTG
1162	383	TCGTGATTATGGATGGCAACGTGAA	1083	TCGCTTGAGTGTAGTCATGATTGCG

1163	662	TTATGGATGGCAACGTGAAACGCGT	1083	TCGCTTGAGTGTAGTCATGATTGCG
1164	422	TCTTTGCCATTGAAGATGACTTAAGC	1083	TCGCTTGAGTGTAGTCATGATTGCG
1165	194	TACTAGCGGTAAGCTTAAACAAGATTGC	1173	TGAGTCGGGTTCACTTTACCTGGCA
1166	684	TTGCCAATGATATTCGTTGGTTAGCAAG	1173	TGAGTCGGGTTCACTTTACCTGGCA
1167	375	TCGGCGAAATCCGTATTCCTGAAAATGA	890	TACCGGAAGCACCAGCGACATTAATAG
1168	182	TACCACTATTAATGTCGCTGGTGCTTC	1195	TGCAACTGAATAGATTGCAGTAAGTTATAAGC
1169	656	TTATAACTTACTGCAATCTATTCAGTTGCTTGGTG	1151	TGAATTATGCAAGAAGTGATCAATTTTCTCACGA
1170	656	TTATAACTTACTGCAATCTATTCAGTTGCTTGGTG	1224	TGCCGTAACTAACATAAGAGAATTATGCAAGAA
1171	618	TGGTTATGTACCAAATACTTTGTCTGAAGATGG	1157	TGACGGCATCGATACCACCGTC
2846	302	TCCAAAAAAATCAGCGCGTACAGTGG	852	TAAAGGATAGCGGTAACTAAATGGCTGAGCCAT
2847	199	TACTTGGTAAATACCACCCACATGGTGA	889	TACCCCAGTTCCCCTGACCTTC
2848	596	TGGTAAATACCACCCACATGGTGAC	1169	TGAGCCATGAGTACCATGGCTTCATAACATGC
2852	150	TAAATCTGCCCGTGTCGTTGGTGAC	1242	TGCTAAAGTCTTGAGCCATACGAACAATGG
2853	166	TAATCGGTAAATATCACCCGCATGGTGAC	1069	TCGATCGAACCGAAGTTACCCTGACC
2854	166	TAATCGGTAAATATCACCCGCATGGTGAC	1168	TGAGCCATACGAACAATGGTTTCATAAACAGC
2922	583	TGGGCGATGCTGCGAAATGGTTAAAAGA	923	TAGTATCACCACGTACACCCGGATCAGT
2972	592	TGGGIGATGCTGCIAAATGGTTAAAAGA	924	TAGTATCACCACGTACICCIGGATCAGT

[419] Analysis of bioagent identifying amplicons obtained using the primers of Table 14B for over 200 samples from Operation Iraqi Freedom resulted in the identification of 50 distinct strain type clusters. The largest cluster, designated strain type 11 (ST11) includes 42 sample isolates, all of which were obtained from US service personnel and Iraqi civilians treated at the 28th Combat Support Hospital in Baghdad. Several of these individuals were also treated on the hospital ship USNS Comfort. These observations are indicative of significant epidemiological correlation/linkage.

[420] All of the sample isolates were tested against a broad panel of antibiotics to characterize their antibiotic resistance profiles. As an example of a representative result from antibiotic susceptibility testing, ST11 was found to consist of four different clusters of isolates, each with a varying degree of sensitivity/resistance to the various antibiotics tested which included penicillins, extended spectrum penicillins, cephalosporins, carbepenem, protein synthesis inhibitors, nucleic acid synthesis inhibitors, anti-metabolites, and anti-cell membrane antibiotics. Thus, the genotyping power of bacterial bioagent identifying amplicons, particularly drill-down bacterial bioagent identifying amplicons, has the potential to increase the understanding of the transmission of infections in combat casualties, to identify the source of infection in the environment, to track hospital transmission of nosocomial infections, and to

rapidly characterize drug-resistance profiles which enable development of effective infection control measures on a time-scale previously not achievable.

Example 13: Triangulation Genotyping Analysis and Codon Analysis of *Acinetobacter baumannii* Samples from Two Health Care Facilities

[421] In this investigation, 88 clinical samples were obtained from Walter Reed Hospital and 95 clinical samples were obtained from Northwestern Medical Center. All samples from both healthcare facilities were suspected of containing sub-types of *Acinetobacter baumannii*, at least some of which were expected to be resistant to quinolone drugs. Each of the 183 samples was analyzed by the method of the present invention. DNA was extracted from each of the samples and amplified with eight triangulation genotyping analysis primer pairs represented by primer pair numbers: 1151, 1156, 1158, 1160, 1165, 1167, 1170, and 1171. The DNA was also amplified with speciating primer pair number 2922 and codon analysis primer pair numbers 2846-2848 which interrogate a codon present in the parC gene, and primer pair numbers 2852-2854 which bracket a codon present in the gyrA gene. The parC and gyrA codon mutations are both responsible for causing drug resistance in *Acinetobacter baumannii*. During evolution of drug resistant strains, the gyrA mutation usually occurs before the parC mutation. Amplification products were measured by ESI-TOF mass spectrometry as indicated in Example 4. The base compositions of the amplification products were calculated from the average molecular masses of the amplification products and are shown in Tables 15-18. The entries in each of the tables are grouped according to strain type number, which is an arbitrary number assigned to *Acinetobacter baumannii* strains in the order of observance beginning from the triangulation genotyping analysis OIF genotyping study described in Example 12. For example, strain type 11 which appears in samples from the Walter Reed Hospital is the same strain as the strain type 11 mentioned in Example 12. Ibis# refers to the order in which each sample was analyzed. Isolate refers to the original sample isolate numbering system used at the location from which the samples were obtained (either Walter Reed Hospital or Northwestern Medical Center). ST = strain type. ND = not detected. Base compositions highlighted with **bold** type indicate that the base composition is a unique base composition for the amplification product obtained with the pair of primers indicated.

Table 15A: Base Compositions of Amplification Products of 88 *A. baumannii* Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the gyrA Gene

Species	Ibis#	Isolate	ST	PP No: 2852 gyrA	PP No: 2853 gyrA	PP No: 2854 gyrA
A. baumannii	20	1082	1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	13	854	10	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	22	1162	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	27	1230	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	31	1367	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	37	1459	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	55	1700	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	64	1777	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	73	1861	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	74	1877	10	ND	A29G28C21T43	A17G13C13T21
A. baumannii	86	1972	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	3	684	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	6	720	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	7	726	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	19	1079	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	21	1123	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	1188	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	33	1417	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	34	1431	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	38	1496	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	40	1523	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	42	1640	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	50	1666	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	51	1668	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	52	1695	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	65	1781	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	44	1649	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49A	1658.1	12	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	49B	1658.2	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	56	1707	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	1893	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	5	693	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	8	749	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	10	839	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	14	865	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	16	888	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	29	1326	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	1440	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	1524	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	46	1652	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	47	1653	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	48	1657	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	1709	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	1727	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	1762	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	1806	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	1881	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	1886	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	1	649	46	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	2	653	46	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	39	1497	16	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	24	1198	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	28	1243	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	43	1648	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	62	1746	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	4	689	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	68	1822	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	69	1823A	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	70	1823B	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	71	1826	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	72	1860	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	81	1924	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	82	1929	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	85	1966	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	11	841	3	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	32	1415	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	45	1651	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	54	1697	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	58	1712	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	60	1725	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	66	1802	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	76	1883	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	78	1891	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	79	1892	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	83	1947	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	84	1964	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	53	1696	24	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	36	1458	49	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	59	1716	9	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	9	805	30	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	18	967	39	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	30	1322	48	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. baumannii	26	1218	50	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 13TU	15	875	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 13TU	17	895	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 3	12	853	B7	A25G22C22T32	A30G29C22T40	A17G13C14T20
A. johnsonii	25	1202	NEW1	A25G22C22T32	A30G29C22T40	A17G13C14T20
A. sp. 2082	87	2082	NEW2	A25G22C22T32	**A31G28C22T40**	A17G13C14T20

Table 15B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the parC Gene

Species	Ibis#	Isolate	ST	PP No: 2846 parC	PP No: 2847 parC	PP No: 2848 parC
A. baumannii	20	1082	1	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	13	854	10	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	22	1162	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	27	1230	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	31	1367	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	37	1459	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	55	1700	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	64	1777	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	73	1861	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	74	1877	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	86	1972	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	3	684	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	6	720	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	7	726	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	19	1079	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	21	1123	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	23	1188	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	33	1417	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	34	1431	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	38	1496	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	40	1523	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	42	1640	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	50	1666	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	51	1668	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	52	1695	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	65	1781	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	44	1649	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	49A	1658.1	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	49B	1658.2	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	56	1707	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	80	1893	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	5	693	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	8	749	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	10	839	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	14	865	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	16	888	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	29	1326	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	35	1440	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	41	1524	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	46	1652	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	47	1653	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	48	1657	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	57	1709	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	61	1727	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	63	1762	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	67	1806	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	75	1881	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	77	1886	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	1	649	46	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	2	653	46	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	39	1497	16	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	24	1198	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	28	1243	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	43	1648	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	62	1746	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
A. baumannii	4	689	15	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	68	1822	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	69	1823A	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	70	1823B	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	71	1826	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	72	1860	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	81	1924	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	82	1929	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	85	1966	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	11	841	3	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	32	1415	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	45	1651	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	54	1697	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	58	1712	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	60	1725	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	66	1802	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	76	1883	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	78	1891	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	79	1892	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	83	1947	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	84	1964	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	53	1696	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	36	1458	49	A34G26C29T32	A30G28C24T32	A16G14C15T15
A. baumannii	59	1716	9	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	9	805	30	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	18	967	39	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	30	1322	48	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	26	1218	50	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. sp. 13TU	15	875	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
A. sp. 13TU	17	895	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
A. sp. 3	12	853	B7	A29G26C27T39	A26G32C21T35	A16G14C15T15
A. johnsonii	25	1202	NEW1	A32G28C26T35	A29G29C22T34	A16G14C15T15
A. sp. 2082	87	2082	NEW2	**A33G27C26T35**	**A31G28C20T35**	A16G14C15T15

Table 16A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the gyrA Gene

Species	Ibis#	Isolate	ST	PP No: 2852 gyrA	PP No: 2853 gyrA	PP No: 2854 gyrA

A. baumannii	54	536	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	87	665	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	8	80	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	9	91	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	10	92	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	11	131	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	12	137	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	21	218	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	26	242	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	94	678	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	1	9	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	2	13	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	3	19	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	4	24	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	5	36	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	6	39	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	13	139	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	15	165	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	16	170	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	17	186	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	20	202	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	22	221	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	24	234	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	25	239	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	33	370	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	34	389	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	19	201	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	27	257	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	29	301	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	31	354	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	36	422	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	37	424	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	38	434	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	39	473	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	40	482	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	44	512	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	45	516	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	47	522	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	48	526	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	50	528	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	52	531	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	53	533	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	56	542	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	59	550	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	62	556	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	64	557	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	70	588	51	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	73	603	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	74	605	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	606	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	611	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	79	622	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	83	643	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	85	653	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	89	669	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	93	674	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	228	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	32	369	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	393	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	30	339	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	485	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	42	493	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	43	502	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	46	520	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49	527	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	51	529	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	65	562	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	68	579	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	546	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	58	548	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	60	552	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	555	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	557	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	66	570	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	578	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	69	584	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	71	593	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	72	602	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	76	609	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	78	621	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	625	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	81	628	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	82	632	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	84	649	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	86	655	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	88	668	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	90	671	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	91	672	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	92	673	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	18	196	55	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	55	537	27	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	28	263	27	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 3	14	164	B7	A25G22C22T32	A30G29C22T40	A17G13C14T20
mixture	7	71	-	ND	ND	**A17G13C15T19**

Table 16B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the parC Gene

Species	Ibis#	Isolate	ST	PP No: 2846 parC	PP No: 2847 parC	PP No: 2848 parC
A. baumannii	54	536	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	87	665	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	8	80	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	9	91	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	10	92	10	A33G26C28T34	A29G28C25T32	ND
A. baumannii	11	131	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	12	137	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	21	218	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	26	242	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	94	678	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	1	9	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	2	13	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	3	19	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	4	24	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	5	36	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	6	39	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	13	139	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	15	165	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	16	170	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	17	186	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	20	202	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	22	221	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	24	234	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	25	239	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	33	370	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	34	389	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	19	201	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	27	257	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	29	301	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	31	354	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	36	422	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	37	424	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	38	434	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	39	473	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	40	482	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	44	512	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	45	516	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	47	522	51	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	48	526	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	50	528	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	52	531	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	53	533	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	56	542	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	59	550	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	62	556	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	64	557	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	70	588	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	73	603	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	74	605	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	75	606	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	77	611	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	79	622	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	83	643	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	85	653	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	89	669	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	93	674	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	23	228	51	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	32	369	52	A34G25C28T34	A30G27C25T32	A16G14C14T16
A. baumannii	35	393	52	A34G25C28T34	A30G27C25T32	A16G14C14T16
A. baumannii	30	339	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	41	485	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	42	493	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	43	502	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	46	520	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	49	527	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	51	529	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	65	562	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	68	579	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	57	546	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	58	548	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	60	552	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	61	555	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	63	557	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	66	570	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	67	578	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	69	584	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	71	593	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	72	602	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	76	609	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	78	621	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	80	625	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	81	628	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	82	632	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	84	649	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	86	655	54	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	88	668	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	90	671	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	91	672	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	92	673	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	18	196	55	A33G27C28T33	A29G28C25T31	A15G14C15T16
A. baumannii	55	537	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. baumannii	28	263	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
A. sp. 3	14	164	B7	A35G25C29T32	A30G28C17T39	A16G14C15T15
mixture	7	71	-	ND	ND	A17G14C15T14

Table 17A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 Adk
A. baumannii	20	1082	1	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	13	854	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	22	1162	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	27	1230	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	31	1367	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	37	1459	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	55	1700	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	64	1777	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	73	1861	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	74	1877	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	86	1972	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	3	684	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	6	720	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	7	726	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	19	1079	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	21	1123	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	23	1188	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	33	1417	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	34	1431	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	38	1496	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	40	1523	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	42	1640	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	50	1666	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	51	1668	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	52	1695	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	65	1781	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	44	1649	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	49A	1658.1	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	49B	1658.2	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	56	1707	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	80	1893	12	A45G34C25T43	A44G35C21T42	A44G32C26T38

A. baumannii	5	693	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	8	749	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	10	839	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	14	865	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	16	888	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	29	1326	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	35	1440	14	A44G35C25T43	ND	A44G32C27T37
A. baumannii	41	1524	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	46	1652	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	47	1653	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	48	1657	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	57	1709	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	61	1727	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	63	1762	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	67	1806	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	75	1881	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	77	1886	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	1	649	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	2	653	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	39	1497	16	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	24	1198	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	28	1243	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	43	1648	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	62	1746	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	4	689	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	68	1822	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	69	1823A	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	70	1823B	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	71	1826	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	72	1860	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	81	1924	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	82	1929	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	85	1966	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	11	841	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	32	1415	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	45	1651	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	54	1697	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	58	1712	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	60	1725	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	66	1802	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	76	1883	24	ND	A43G36C20T43	A44G32C27T37
A. baumannii	78	1891	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	79	1892	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	83	1947	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	84	1964	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	53	1696	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	36	1458	49	A44G35C25T43	A44G35C22T41	A44G32C27T37

A. baumannii	59	1716	9	A44G35C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	9	805	30	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	18	967	39	A45G34C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	30	1322	48	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	26	1218	50	A44G35C25T43	A44G35C21T42	A44G32C26T38
A. sp. 13TU	15	875	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
A. sp. 13TU	17	895	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
A. sp. 3	12	853	B7	A46G35C24T42	**A42G34C20T46**	A43G33C24T40
A. johnsonii	25	1202	NEW1	A46G35C23T43	**A42G35C21T44**	**A43G33C23T41**
A. sp. 2082	87	2082	NEW2	**A46G36C22T43**	**A42G32C20T48**	**A42G34C23T41**

Table 17B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1158 and 1160 and 1165

Species	Ibis#	Isolate	ST	PP No: 1158 mutY	PP No: 1160 mutY	PP No: 1165 fumC
A. baumannii	20	1082	1	A27G21C25T22	A32G35C29T33	A40G33C30T36
A. baumannii	13	854	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	22	1162	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	27	1230	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	31	1367	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	37	1459	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	55	1700	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	64	1777	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	73	1861	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	74	1877	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	86	1972	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	3	684	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	6	720	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	7	726	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	19	1079	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	21	1123	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	23	1188	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	33	1417	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	34	1431	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	38	1496	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	40	1523	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	42	1640	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	50	1666	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	51	1668	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	52	1695	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	65	1781	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
A. baumannii	44	1649	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49A	1658.1	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49B	1658.2	12	A27G21C26T21	A32G34C29T34	A40G33C30T36

A. baumannii	56	1707	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	80	1893	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	5	693	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	8	749	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	10	839	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	14	865	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	16	888	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	29	1326	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	35	1440	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	41	1524	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	46	1652	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	47	1653	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	48	1657	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	57	1709	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	61	1727	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	63	1762	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	67	1806	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	75	1881	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	77	1886	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	1	649	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	2	653	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	39	1497	16	A29G19C26T21	A31G35C29T34	A40G34C29T36
A. baumannii	24	1198	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	28	1243	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	43	1648	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	62	1746	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	4	689	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	68	1822	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	69	1823A	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	70	1823B	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	71	1826	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	72	1860	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	81	1924	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	82	1929	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	85	1966	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	11	841	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	32	1415	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	45	1651	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	54	1697	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	58	1712	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	60	1725	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	66	1802	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	76	1883	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	78	1891	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	79	1892	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	83	1947	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	84	1964	24	A27G21C26T21	A32G35C28T34	A40G33C30T36

A. baumannii	53	1696	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	36	1458	49	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	59	1716	9	A27G21C25T22	A32G35C28T34	A39G33C30T37
A. baumannii	9	805	30	A27G21C25T22	A32G35C28T34	A39G33C30T37
A. baumannii	18	967	39	A27G21C26T21	A32G35C28T34	A39G33C30T37
A. baumannii	30	1322	48	A28G21C24T22	A32G35C29T33	A40G33C30T36
A. baumannii	26	1218	50	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. sp. 13TU	15	875	A1	A27G21C25T22	**A30G36C26T37**	A41G34C28T36
A. sp. 13TU	17	895	A1	A27G21C25T22	**A30G36C26T37**	A41G34C28T36
A. sp. 3	12	853	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
A. johnsonii	25	1202	NEW1	**A25G23C24T23**	A30G35C30T34	**A38G37C26T38**
A. sp. 2082	87	2082	NEW2	**A26G22C24T23**	A31G35C28T35	**A42G34C27T36**

Table 17C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1167 and 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
A. baumannii	20	1082	1	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	13	854	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	22	1162	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	27	1230	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	31	1367	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	37	1459	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	55	1700	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	64	1777	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	73	1861	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	74	1877	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	86	1972	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	3	684	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	6	720	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	7	726	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	19	1079	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	21	1123	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	23	1188	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	33	1417	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	34	1431	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	38	1496	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	40	1523	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	42	1640	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	50	1666	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	51	1668	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	52	1695	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	65	1781	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	44	1649	12	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	49A	1658.1	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	49B	1658.2	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	56	1707	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	80	1893	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	693	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	8	749	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	10	839	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	14	865	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	16	888	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	1326	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	35	1440	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	41	1524	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	46	1652	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	47	1653	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	48	1657	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	57	1709	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	61	1727	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	63	1762	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	67	1806	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	75	1881	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	77	1886	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	1	649	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	2	653	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	39	1497	16	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	24	1198	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	28	1243	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	43	1648	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	62	1746	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	4	689	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	68	1822	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	69	1823A	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	70	1823B	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	71	1826	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	72	1860	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	81	1924	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	82	1929	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	85	1966	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	11	841	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	32	1415	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	45	1651	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	54	1697	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	58	1712	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	60	1725	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	66	1802	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	76	1883	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	78	1891	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	79	1892	24	A40G35C34T38	A39G26C22T49	A35G37C33T44

A. baumannii	83	1947	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	84	1964	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	53	1696	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	36	1458	49	A40G35C34T38	A39G26C22T49	A35G37C30T47
A. baumannii	59	1716	9	A40G35C32T40	A38G27C20T51	**A36G35C31T47**
A. baumannii	9	805	30	A40G35C32T40	A38G27C21T50	A35G36C29T49
A. baumannii	18	967	39	A40G35C33T39	A38G27C20T51	A35G37C30T47
A. baumannii	30	1322	48	A40G35C35T37	A38G27C21T50	A35G37C30T47
A. baumannii	26	1218	50	A40G35C34T38	A38G27C21T50	A35G37C33T44
A. sp. 13TU	15	875	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
A. sp. 13TU	17	895	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
A. sp. 3	12	853	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
A. johnsonii	25	1202	NEW1	**A42G38C31T36**	**A40G27C19T50**	A35G37C32T45
A. sp. 2082	87	2082	NEW2	**A43G37C32T35**	**A37G26C21T52**	**A35G38C31T45**

Table 18A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 adk
A. baumannii	54	536	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	87	665	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	8	80	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	9	91	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	10	92	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	11	131	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	12	137	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	21	218	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	26	242	10	A45G34C25T43	**A44G35C21T42**	A44G32C26T38
A. baumannii	94	678	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	1	9	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	2	13	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	3	19	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	4	24	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	5	36	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	6	39	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	13	139	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	15	165	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	16	170	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	17	186	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	20	202	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	22	221	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	24	234	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	25	239	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	33	370	10	A45G34C25T43	A44G35C21T42	A44G32C26T38

A. baumannii	34	389	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	19	201	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	27	257	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	29	301	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	31	354	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	36	422	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	37	424	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	38	434	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	39	473	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	40	482	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	44	512	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	45	516	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	47	522	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	48	526	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	50	528	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	52	531	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	53	533	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	56	542	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	59	550	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	62	556	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	64	557	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	70	588	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	73	603	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	74	605	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	75	606	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	77	611	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	79	622	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	83	643	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	85	653	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	89	669	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	93	674	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	23	228	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	32	369	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	35	393	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	30	339	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	41	485	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	42	493	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	43	502	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	46	520	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	49	527	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	51	529	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	65	562	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	68	579	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	57	546	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	58	548	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	60	552	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	61	555	54	A44G35C25T43	A44G35C20T43	A44G32C26T38

A. baumannii	63	557	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	66	570	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	67	578	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	69	584	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	71	593	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	72	602	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	76	609	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	78	621	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	80	625	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	81	628	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	82	632	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	84	649	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	86	655	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	88	668	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	90	671	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	91	672	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	92	673	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	18	196	55	A44G35C25T43	A44G35C20T43	A44G32C27T37
A. baumannii	55	537	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	28	263	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. sp. 3	14	164	B7	A46G35C24T42	**A42G34C20T46**	A43G33C24T40
mixture	7	71	?	mixture	ND	ND

Table 18B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1158, 1160 and 1165

Species	Ibis#	Isolate	ST	PP No: 1158 mutY	PP No: 1160 mutY	PP No: 1165 fumC
A. baumannii	54	536	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	87	665	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	8	80	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	9	91	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	10	92	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	11	131	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	12	137	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	21	218	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	26	242	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	94	678	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	1	9	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	2	13	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	3	19	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	4	24	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	5	36	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	6	39	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	13	139	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	15	165	10	A27G21C26T21	A32G35C28T34	A40G33C30T36

A. baumannii	16	170	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	17	186	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	20	202	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	22	221	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	24	234	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	25	239	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	33	370	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	34	389	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	19	201	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	27	257	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	29	301	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	31	354	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	36	422	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	37	424	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	38	434	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	39	473	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	40	482	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	44	512	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	45	516	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	47	522	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	48	526	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	50	528	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	52	531	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	53	533	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	56	542	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	59	550	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	62	556	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	64	557	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	70	588	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	73	603	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	74	605	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	75	606	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	77	611	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	79	622	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	83	643	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	85	653	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	89	669	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	93	674	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	23	228	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	32	369	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	35	393	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	30	339	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	41	485	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	42	493	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	43	502	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	46	520	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	49	527	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36

A. baumannii	51	529	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	65	562	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	68	579	53	**A28G20C26T21**	A32G34C29T34	A40G33C30T36
A. baumannii	57	546	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	58	548	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	60	552	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	61	555	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	63	557	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	66	570	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	67	578	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	69	584	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	71	593	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	72	602	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	76	609	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	78	621	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	80	625	54	A27G21C26T21	A32G34C29T34	**A40G33C30T36**
A. baumannii	81	628	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	82	632	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	84	649	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	86	655	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	88	668	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	90	671	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	91	672	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	92	673	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	18	196	55	A27G21C25T22	**A31G36C27T35**	A40G33C29T37
A. baumannii	55	537	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. baumannii	28	263	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. sp. 3	14	164	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
mixture	7	71	?	ND	ND	ND

Table 18C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1167, 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
A. baumannii	54	536	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	87	665	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	8	80	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	9	91	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	10	92	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	11	131	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	12	137	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	21	218	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	26	242	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	94	678	10	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	1	9	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	2	13	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	3	19	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	4	24	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	36	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	6	39	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	13	139	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	15	165	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	16	170	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	17	186	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	20	202	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	22	221	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	24	234	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	25	239	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	33	370	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	34	389	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	19	201	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	27	257	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	301	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	31	354	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	36	422	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	37	424	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	38	434	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	39	473	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	40	482	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	44	512	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	45	516	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	47	522	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	48	526	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	50	528	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	52	531	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	53	533	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	56	542	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	59	550	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	62	556	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	64	557	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	70	588	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	73	603	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	74	605	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	75	606	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	77	611	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	79	622	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	83	643	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	85	653	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	89	669	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	93	674	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	23	228	51	A40G35C34T38	A38G27C21T50	A35G37C30T47

A. baumannii	32	369	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
A. baumannii	35	393	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
A. baumannii	30	339	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	41	485	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	42	493	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	43	502	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	46	520	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	49	527	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	51	529	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	65	562	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	68	579	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
A. baumannii	57	546	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	58	548	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	60	552	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	61	555	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	63	557	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	66	570	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	67	578	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	69	584	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	71	593	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	72	602	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	76	609	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	78	621	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	80	625	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	81	628	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	82	632	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	84	649	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	86	655	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	88	668	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	90	671	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	91	672	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	92	673	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
A. baumannii	18	196	55	A42G34C33T38	A38G27C20T51	A35G37C31T46
A. baumannii	55	537	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
A. baumannii	28	263	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
A. sp. 3	14	164	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
mixture	7	71	-	ND	ND	ND

[422] Base composition analysis of the samples obtained from Walter Reed hospital indicated that a majority of the strain types identified were the same strain types already characterized by the OIF study of Example 12. This is not surprising since at least some patients from which clinical samples were obtained in OIF were transferred to the Walter Reed Hospital (WRAIR). Examples of these common strain types include: ST10, ST11, ST12, ST14, ST15, ST16 and ST46. A strong correlation was noted between these strain types and the presence of mutations in the gyrA and parC which confer quinolone drug resistance.

[423] In contrast, the results of base composition analysis of samples obtained from Northwestern Medical Center indicate the presence of 4 major strain types: ST10, ST51, ST53 and ST54. All of these strain types have the gyrA quinolone resistance mutation and most also have the parC quinolone resistance mutation, with the exception of ST35. This observation is consistent with the current understanding that the gyrA mutation generally appears before the parC mutation and suggests that the acquisition of these drug resistance mutations is rather recent and that resistant isolates are taking over the wild-type isolates. Another interesting observation was that a single isolate of ST3 (isolate 841) displays a triangulation genotyping analysis pattern similar to other isolates of ST3, but the codon analysis amplification product base compositions indicate that this isolate has not yet undergone the quinolone resistance mutations in gyrA and parC.

[424] The six isolates that represent species other than *Acinetobacter baumannii* in the samples obtained from the Walter Reed Hospital were each found to not carry the drug resistance mutations.

[425] The results described above involved analysis of 183 samples using the methods and compositions of the present invention. Results were provided to collaborators at the Walter Reed hospital and Northwestern Medical center within a week of obtaining samples. This example highlights the rapid throughput characteristics of the analysis platform and the resolving power of triangulation genotyping analysis and codon analysis for identification of and determination of drug resistance in bacteria.

Example 14: Identification of Drug Resistance Genes and Virulence Factors in *Staphylococcus aureus*

[426] An eight primer pair panel was designed for identification of drug resistance genes and virulence factors of *Staphylococcus aureus* and is shown in Table 19. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 19.

Table 19: Primer Pairs for Identification of Drug Resistance Genes and Virulence Factors in *Staphylococcus aureus*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
879	MECA_Y14051_4507_4530_F	288	MECA_Y14051_4555_4581_R	1269	mecA
2056	MECI-R_NC003923-41798-41609_33_60_F	698	MECI-R_NC003923-41798-41609_86_113_R	1420	MecI-R
2081	ERMA_NC002952-55890-	217	ERMA_NC002952-55890-	1167	ermA

	56621_366_395_F		56621_438_465_R		
2086	ERMC_NC005908-2004-2738_85_116_F	399	ERMC_NC005908-2004-2738_173_206_R	1041	ermC
2095	PVLUK_NC003923-1529595-1531285_688_713_F	456	PVLUK_NC003923-1529595-1531285_775_804_R	1261	Pv-luk
2249	TUFB_NC002758-615038-616222_696_725_F	430	TUFB_NC002758-615038-616222_793_820_R	1321	tufB
2256	NUC_NC002758-894288-894974_316_345_F	174	NUC_NC002758-894288-894974_396_421_R	853	Nuc
2313	MUPR_X75439_2486_2516_F	172	MUPR_X75439_2548_2574_R	1360	mupR

[427] Primer pair numbers 2256 and 2249 are confirmation primers designed with the aim of high level identification of *Staphylococcus aureus*. The nuc gene is a *Staphylococcus aureus*-specific marker gene. The tufB gene is a universal housekeeping gene but the bioagent identifying amplicon defined by primer pair number 2249 provides a unique base composition (A43 G28 C19 T35) which distinguishes *Staphylococcus aureus* from other members of the genus *Staphylococcus*.

[428] High level methicillin resistance in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair numbers 879 and 2056. Analyses have indicated that primer pair number 879 is not expected to prime *S. sciuri* homolog or *Enterococcus faecalis/faciem* ampicillin-resistant PBP5 homologs.

[429] Macrolide and erythromycin resistance in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair numbers 2081 and 2086.

[430] Resistance to mupriocin in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair number 2313.

[431] Virulence in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair number 2095. This primer pair can simultaneously and identify the pvl (lukS-PV) gene and the lukD gene which encodes a homologous enterotoxin. A bioagent identifying amplicon of the lukD gene has a six nucleobase length difference relative to the lukS-PV gene.

[432] A total of 32 blinded samples of different strains of *Staphylococcus aureus* were provided by the Center for Disease Control (CDC). Each sample was analyzed by PCR amplification with the eight primer pair panel, followed by purification and measurement of molecular masses of the amplification products by mass spectrometry. Base compositions for the amplification products were calculated. The base compositions provide the information summarized above for each primer pair. The results are shown in Tables 20A and B. One result noted upon un-blinding of the samples is that each of the PVL+ identifications agreed with PVL+ identified in the same samples by standard PCR assays. These results

indicate that the panel of eight primer pairs is useful for identification of drug resistance and virulence sub-species characteristics for *Staphylococcus aureus*. It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Table 20A: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2081, 2086, 2095 and 2256

Sample Index No.	Primer Pair No. 2081 (ermA)	Primer Pair No. 2086 (ermC)	Primer Pair No. 2095 (pv-luk)	Primer Pair No. 2256 (nuc)
CDC0010	-	-	PVL-/lukD+	+
CDC0015	-	-	PVL+/lukD+	+
CDC0019	-	+	PVL-/lukD+	+
CDC0026	+	-	PVL-/lukD+	+
CDC0030	+	-	PVL-/lukD+	+
CDC004	-	-	PVL+/lukD+	+
CDC0014	-	+	PVL+/lukD+	+
CDC008	-	-	PVL-/lukD+	+
CDC001	+	-	PVL-/lukD+	+
CDC0022	+	-	PVL-/lukD+	+
CDC006	+	-	PVL-/lukD+	+
CDC007	-	-	PVL-/lukD+	+
CDCVRSA1	+	-	PVL-/lukD+	+
CDCVRSA2	+	+	PVL-/lukD+	+
CDC0011	+	-	PVL-/lukD+	+
CDC0012	-	-	PVL+/lukD-	+
CDC0021	+	-	PVL-/lukD+	+
CDC0023	+	-	PVL-/lukD+	+
CDC0025	+	-	PVL-/lukD+	+
CDC005	-	-	PVL-/lukD+	+
CDC0018	+	-	PVL+/lukD-	+
CDC002	-	-	PVL-/lukD+	+
CDC0028	+	-	PVL-/lukD+	+
CDC003	-	-	PVL-/lukD+	+
CDC0013	-	-	PVL+/lukD+	+
CDC0016	-	-	PVL-/lukD+	+
CDC0027	+	-	PVL-/lukD+	+
CDC0029	-	-	PVL+/lukD+	+

CDC0020	-	+	PVL-/lukD+	+
CDC0024	-	-	PVL-/lukD+	+
CDC0031	-	-	PVL-/lukD+	+

Table 20B: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2249, 879, 2056, and 2313

Sample Index No.	Primer Pair No. 2249 (tufB)	Primer Pair No. 879 (mecA)	Primer Pair No. 2056 (mecI-R)	Primer Pair No. 2313 (mupR)
CDC0010	*Staphylococcus aureus*	+	+	-
CDC0015	*Staphylococcus aureus*	-	-	-
CDC0019	*Staphylococcus aureus*	+	+	-
CDC0026	*Staphylococcus aureus*	+	+	-
CDC0030	*Staphylococcus aureus*	+	+	-
CDC004	*Staphylococcus aureus*	+	+	-
CDC0014	*Staphylococcus aureus*	+	+	-
CDC008	*Staphylococcus aureus*	+	+	-
CDC001	*Staphylococcus aureus*	+	+	-
CDC0022	*Staphylococcus aureus*	+	+	-
CDC006	*Staphylococcus aureus*	+	+	+
CDC007	*Staphylococcus aureus*	+	+	-
CDCVRSA1	*Staphylococcus aureus*	+	+	-
CDCVRSA2	*Staphylococcus aureus*	+	+	-
CDC0011	*Staphylococcus aureus*	-	-	-
CDC0012	*Staphylococcus aureus*	+	+	-
CDC0021	*Staphylococcus aureus*	+	+	-
CDC0023	*Staphylococcus aureus*	+	+	-
CDC0025	*Staphylococcus aureus*	+	+	-
CDC005	*Staphylococcus aureus*	+	+	-
CDC0018	*Staphylococcus aureus*	+	+	-
CDC002	*Staphylococcus aureus*	+	+	-
CDC0028	*Staphylococcus aureus*	+	+	-
CDC003	*Staphylococcus aureus*	+	+	-
CDC0013	*Staphylococcus aureus*	+	+	-
CDC0016	*Staphylococcus aureus*	+	+	-
CDC0027	*Staphylococcus aureus*	+	+	-
CDC0029	*Staphylococcus aureus*	+	+	-

CDC0020	*Staphylococcus aureus*	-	-	-
CDC0024	*Staphylococcus aureus*	+	+	-
CDC0031	*Staphylococcus scleiferi*	-	-	-

Example 15: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for *Staphylococcus aureus*

[433] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within six different housekeeping genes which are listed in Table 21. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 21.

Table 21: Primer Pairs for Triangulation Genotyping Analysis of *Staphylococcus aureus*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
2146	ARCC_NC003923-2725050-2724595_131_161_F	437	ARCC_NC003923-2725050-2724595_214_245_R	1137	arcC
2149	AROE_NC003923-1674726-1674277_30_62_F	530	AROE_NC003923-1674726-1674277_155_181_R	891	aroE
2150	AROE_NC003923-1674726-1674277_204_232_F	474	AROE_NC003923-1674726-1674277_308_335_R	869	aroE
2156	GMK_NC003923-1190906-1191334_301_329_F	268	GMK_NC003923-1190906-1191334_403_432_R	1284	gmk
2157	PTA_NC003923-628885-629355_237_263_F	418	PTA_NC003923-628885-629355_314_345_R	1301	pta
2161	TPI_NC003923-830671-831072_1_34_F	318	TPI_NC003923-830671-831072_97_129_R	1300	tpi
2163	YQI_NC003923-378916-379431_142_167_F	440	YQI_NC003923-378916-379431_259_284_R	1076	yqi
2166	YQI_NC003923-378916-379431_275_300_F	219	YQI_NC003923-378916-379431_364_396_R	1013	yqi

[434] The same samples analyzed for drug resistance and virulence in Example 14 were subjected to triangulation genotyping analysis. The primer pairs of Table 21 were used to produce amplification products by PCR, which were subsequently purified and measured by mass spectrometry. Base compositions were calculated from the molecular masses and are shown in Tables 22A and 22B.

Table 22A: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample Index No.	Strain	Primer Pair No. 2146 (arcC)	Primer Pair No. 2149(aroE)	Primer Pair No. 2150 (aroE)	Primer Pair No. 2156 (gmk)
CDC0010	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32

CDC0015	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0019	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0026	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0030	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC004	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0014	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC008	????	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC001	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0022	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC006	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0011	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0012	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0021	MRSA252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0023	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0025	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC005	ST:338	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC0018	ST:338	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC002	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
CDC0028	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
CDC003	ST:107	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0013	ST:12	ND	A59 G24 C18 T51	A40 G36 C13 T43	A51 G29 C21 T31
CDC0016	ST:120	A45 G23 C18 T29	A58 G24 C19 T51	A40 G37 C13 T42	A51 G29 C21 T31
CDC0027	ST:105	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0029	MSSA476	A45 G23 C20 T27	A58 G24 C19 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0020	ST:15	A44 G23 C21 T27	A59 G23 C18 T52	A40 G36 C13 T43	A50 G30 C20 T32
CDC0024	ST:137	A45 G23 C20 T27	A57 G25 C19 T51	A40 G36 C13 T43	A51 G29 C22 T30
CDC0031	***	No product	No product	No product	No product

Table 22B: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample Index No.	Strain	Primer Pair No. 2157 (pta)	Primer Pair No. 2161 (tpi)	Primer Pair No. 2163 (yqi)	Primer Pair No. 2166 (yqi)
CDC0010	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0015	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0019	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0026	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37

CDC0030	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC004	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0014	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC008	unknown	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC001	Mu50	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC0022	Mu50	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC006	Mu50	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC0011	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0012	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0021	MRSA252	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0023	ST:110	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0025	ST:110	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC005	ST:338	A32 G25 C24 T28	A51 G27 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37
CDC0018	ST:338	A32 G25 C24 T28	A51 G27 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37
CDC002	ST:108	A33 G25 C23 T28	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC0028	ST:108	A33 G25 C23 T28	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37
CDC003	ST:107	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0013	ST:12	A32 G25 C23 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37
CDC0016	ST:120	A32 G25 C24 T28	A50 G28 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37
CDC0027	ST:105	A33 G25 C22 T29	A50 G28 C22 T29	A43 G36 C21 T43	A36 G31 C19 T36
CDC0029	MSSA476	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36
CDC0020	ST:15	A33 G25 C22 T29	A50 G28 C21 T30	A42 G36 C22 T43	A36 G31 C18 T37
CDC0024	ST:137	A33 G25 C22 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37
CDC0031	***	A34 G25 C25 T25	A51 G27 C24 T27	No product	No product

[435] Note: *** The sample CDC0031 was identified as *Staphylococcus scleiferi* as indicated in Example 14. Thus, the triangulation genotyping primers designed for *Staphylococcus aureus* would generally not be expected to prime and produce amplification products of this organism. Tables 22A and 22B indicate that amplification products are obtained for this organism only with primer pair numbers 2157 and 2161.

[436] A total of thirteen different genotypes of *Staphylococcus aureus* were identified according to the unique combinations of base compositions across the eight different bioagent identifying amplicons obtained with the eight primer pairs. These results indicate that this eight primer pair panel is useful for analysis of unknown or newly emerging strains of *Staphylococcus aureus*. It is expected that a kit

comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 16: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Vibrio*

[437] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 23. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 23.

Table 23: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus *Vibrio*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1098	RNASEP_VBC_331_349_F	325	RNASEP_VBC_388_414_R	1163	RNAse P
2000	CTXB_NC002505_46_70_F	278	CTXB_NC002505_132_162_R	1039	ctxB
2001	FUR_NC002505_87_113_F	465	FUR_NC002505_205_228_R	1037	fur
2011	GYRB_NC002505_1161_1190_F	148	GYRB_NC002505_1255_1284_R	1172	gyrB
2012	OMPU_NC002505_85_110_F	190	OMPU_NC002505_154_180_R	1254	ompU
2014	OMPU_NC002505_431_455_F	266	OMPU_NC002505_544_567_R	1094	ompU
2323	CTXA_NC002505-1568114-1567341_122_149_F	508	CTXA_NC002505-1568114-1567341_186_214_R	1297	ctxA
2927	GAPA_NC002505_694_721_F	259	GAPA_NC_002505_29_58_R	1060	gapA

[438] A group of 50 bacterial isolates containing multiple strains of both environmental and clinical isolates of *Vibrio cholerae*, 9 other *Vibrio* species, and 3 species of Photobacteria were tested using this panel of primer pairs. Base compositions of amplification products obtained with these 8 primer pairs were used to distinguish amongst various species tested, including sub-species differentiation within *Vibrio cholerae* isolates. For instance, the non-O1/non-O139 isolates were clearly resolved from the O1 and the O139 isolates, as were several of the environmental isolates of *Vibrio cholerae* from the clinical isolates.

[439] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 17: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Pseudomonas*

[440] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of twelve triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 24. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 24.

Table 24: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus *Pseudomonas*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
2949	ACS_NC002516-970624-971013_299_316_F	376	ACS_NC002516-970624-971013_364_383_R	1265	acsA
2950	ARO_NC002516-26883-27380_4_26_F	267	ARO_NC002516-26883-27380_111_128_R	1341	aroE
2951	ARO_NC002516-26883-27380_356_377_F	705	ARO_NC002516-26883-27380_459_484_R	1056	aroE
2954	GUA_NC002516-4226546-4226174_155_178_F	710	GUA_NC002516-4226546-4226174_265_287_R	1259	guaA
2956	GUA_NC002516-4226546-4226174_242_263_F	374	GUA_NC002516-4226546-4226174_355_371_R	1111	guaA
2957	MUT_NC002516-5551158-5550717_5_26_F	545	MUT_NC002516-5551158-5550717_99_116_R	978	mutL
2959	NUO_NC002516-2984589-2984954_8_26_F	249	NUO_NC002516-2984589-2984954_97_117_R	1095	nuoD
2960	NUO_NC002516-2984589-2984954_218_239_F	195	NUO_NC002516-2984589-2984954_301_326_R	1376	nuoD
2961	PPS_NC002516-1915014-1915383_44_63_F	311	PPS_NC002516-1915014-1915383_140_165_R	1014	pps
2962	PPS_NC002516-1915014-1915383_240_258_F	365	PPS_NC002516-1915014-1915383_341_360_R	1052	pps
2963	TRP_NC002516-671831-672273_24_42_F	527	TRP_NC002516-671831-672273_131_150_R	1071	trpE
2964	TRP_NC002516-671831-672273_261_282_F	490	TRP_NC002516-671831-672273_362_383_R	1182	trpE

[441] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

[442] The present invention includes any combination of the various species and subgeneric groupings falling within the generic disclosure. This invention therefore includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[443] While in accordance with the patent statutes, description of the various embodiments and examples have been provided, the scope of the invention is not to be limited thereto or thereby.

Modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

[444] Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims, rather than by the specific examples which have been presented by way of example.

[445] Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank gi or accession numbers, internet web sites, and the like) cited in the present application is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 456.

2. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 1261.

3. A composition comprising the primer of claim 1.

4. The composition of claim 3 further comprising an oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 1261.

5. The composition of claim 4 wherein either or both of said primers comprises at least one modified nucleobase.

6. The composition of claim 5 wherein said modified nucleobase is 5-propynyluracil or 5-propynylcytosine.

7. The composition of claim 4 wherein either or both of said primers comprises at least one universal nucleobase.

8. The composition of claim 7 wherein said universal nucleobase is inosine.

9. The composition of claim 4 wherein either or both of said primers further comprises a non-templated T residue on the 5'-end.

10. The composition of claim 4 wherein either or both of said primers comprises at least one non-template tag.

11. The composition of claim 4 wherein either or both of said primers comprises at least one molecular mass modifying tag.

12. A kit comprising the composition of claim 4.

13. The kit of claim 12 further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.

14. The kit of claim 12 further comprising one or more calibration polynucleotides.

15. The kit of claim 12 further comprising at least one anion exchange functional group linked to a magnetic bead.

16. A method for identification of a strain of *Staphylococcus aureus* in a sample comprising:

amplifying nucleic acid from said strain of *Staphylococcus aureus* using the composition of claim 4 to obtain an amplification product;

determining the molecular mass of said amplification product;

optionally, determining the base composition of said amplification product from said molecular mass; and

comparing said molecular mass or said base composition with a plurality of molecular masses or base compositions of known amplification products of strains of *Staphylococcus aureus* defined by the composition of claim 4, wherein a match between said molecular mass or base composition and a member of said plurality of molecular masses or base compositions identifies said strain of *Staphylococcus aureus*.

17. The method of claim 16 further comprising repeating said amplifying, determining, optionally determining, and comparing steps using at least one additional primer pair, wherein each member of said at least one additional primer pair is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.

18. The method of claim 16 wherein said strain of *Staphylococcus aureus* is a virulent strain.

19. The method of claim 18 wherein said strain of *Staphylococcus aureus* is a virulent strain.

20. A method for determination of the quantity of a strain of *Staphylococcus aureus* in a sample comprising:

contacting said sample with the composition of claim 4 and a known quantity of a calibration polynucleotide comprising a calibration sequence;

concurrently amplifying nucleic acid from said a strain of *Staphylococcus aureus* and nucleic acid from said calibration polynucleotide in said sample with the composition of claim 4 to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon;

determining the molecular mass and abundance for said bacterial bioagent identifying amplicon and said calibration amplicon; and

distinguishing said bacterial bioagent identifying amplicon from said calibration amplicon based on molecular mass, wherein comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of said strain of *Staphylococcus aureus* in said sample.

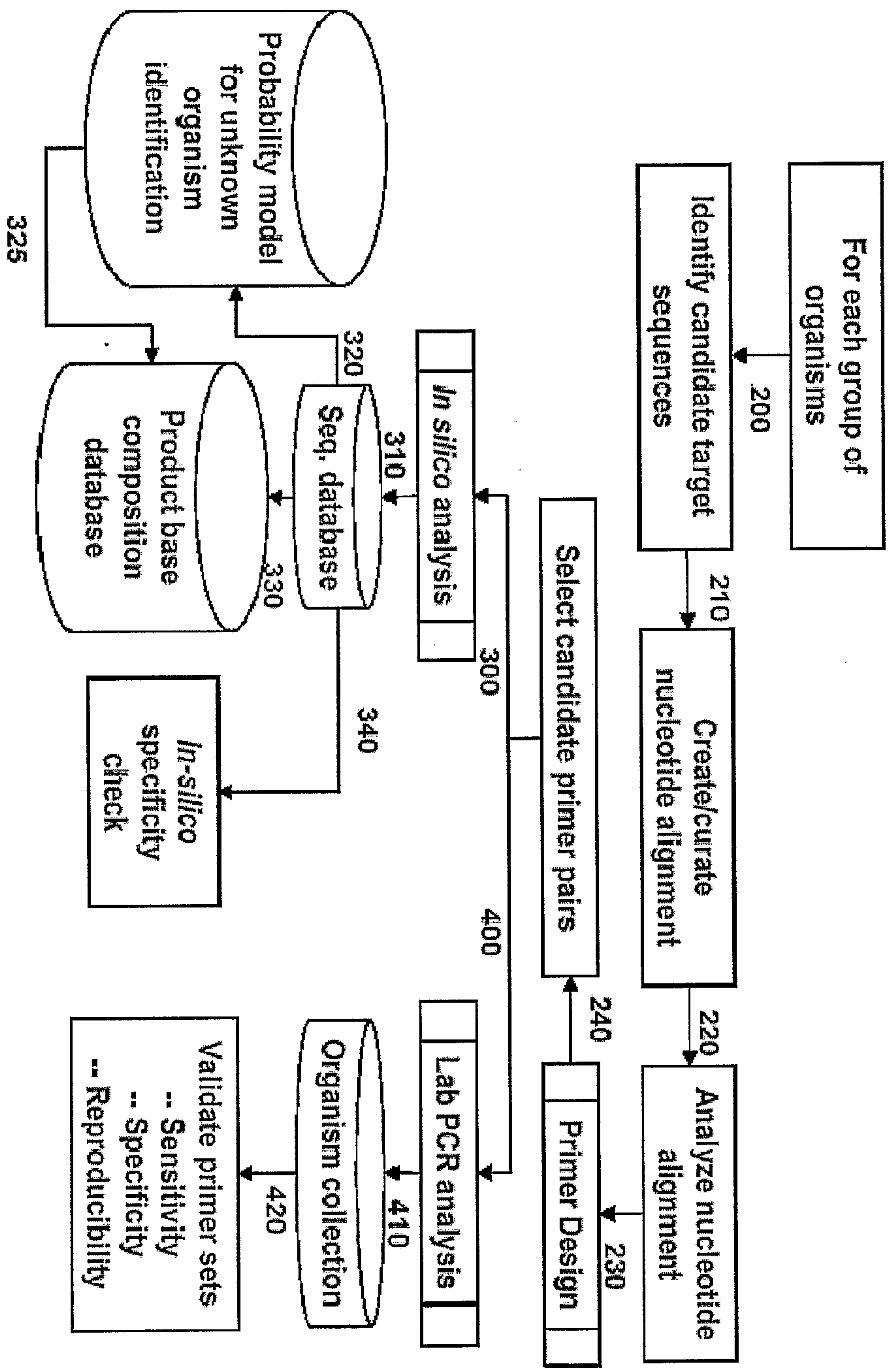

For each group of organisms
200
Identify candidate target sequences
210
Create/curate nucleotide alignment
220
Analyze nucleotide alignment
230
Primer Design
240
Select candidate primer pairs
300
In silico analysis
310
Seq. database
320
Probability model for unknown organism identification
325
330
Product base composition database
340
In-silico specificity check
400
Lab PCR analysis
410
Organism collection
420
Validate primer sets
-- Sensitivity
-- Specificity
-- Reproducibility

Figure 1

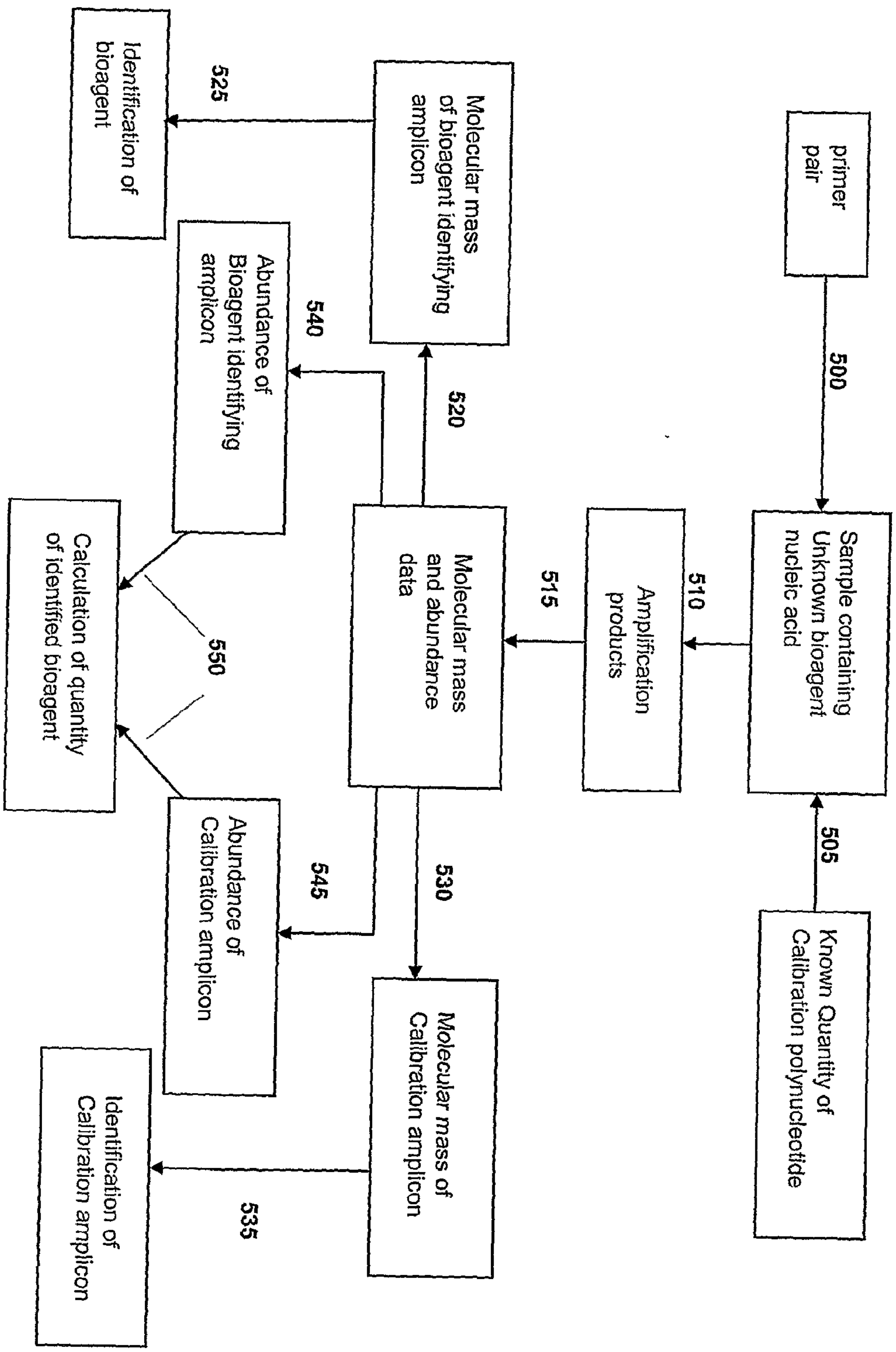

primer pair
500
Sample containing Unknown bioagent nucleic acid
505
Known Quantity of Calibration polynucleotide
510
Amplification products
515
Molecular mass and abundance data
520
Molecular mass of bioagent identifying amplicon
525
Identification of bioagent
530
Molecular mass of Calibration amplicon
535
Identification of Calibration amplicon
540
Abundance of Bioagent identifying amplicon
545
Abundance of Calibration amplicon
550
Calculation of quantity of identified bioagent

Figure 2

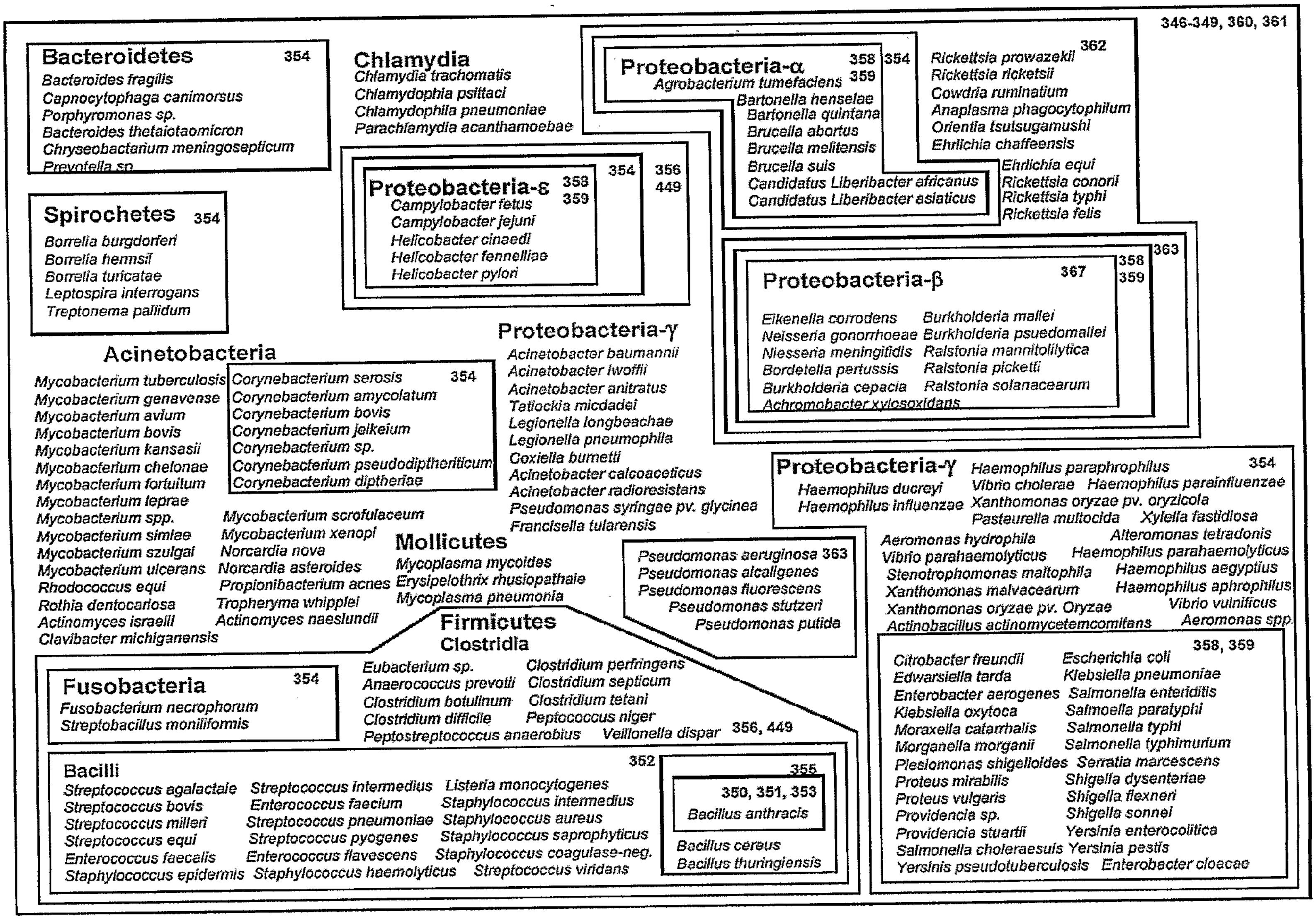

346-349, 360, 361
Bacteroidetes 354
Bacteroides fragilis
Capnocytophaga canimorsus
Porphyromonas sp.
Bacteroides thetaiotaomicron
Chryseobactarium meningosepticum
Prevotella sp
Spirochetes 354
Borrelia burgdorferi
Borrelia hermsii
Borrelia turicatae
Leptospira interrogans
Treptonema pallidum
Chlamydia
Chlamydia trachomatis
Chlamydophila psittaci
Chlamydophila pneumoniae
Parachlamydia acanthamoebae
Proteobacteria-ε 358 359 354 356 449
Campylobacter fetus
Campylobacter jejuni
Helicobacter cinaedi
Helicobacter fennelliae
Helicobacter pylori
Proteobacteria-α 358 359 354
Agrobacterium tumefaciens
Bartonella henselae
Bartonella quintana
Brucella abortus
Brucella melitensis
Brucella suis
Candidatus Liberibacter africanus
Candidatus Liberibacter asiaticus
362
Rickettsia prowazekii
Rickettsia ricketsii
Cowdria ruminatium
Anaplasma phagocytophilum
Orientia tsutsugamushi
Ehrlichia chaffeensis
Ehrlichia equi
Rickettsia conorii
Rickettsia typhi
Rickettsia felis
363
Proteobacteria-β 367 358 359
Eikenella corrodens
Burkholderia mallei
Neisseria gonorrhoeae
Burkholderia psuedomallei
Niesseria meningitidis
Ralstonia mannitolilytica
Bordetella pertussis
Ralstonia picketti
Burkholderia cepacia
Ralstonia solanacearum
Achromobacter xylosoxidans
Acinetobacteria
Mycobacterium tuberculosis
Mycobacterium genavense
Mycobacterium avium
Mycobacterium bovis
Mycobacterium kansasii
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium leprae
Mycobacterium spp.
Mycobacterium simiae
Mycobacterium szulgai
Mycobacterium ulcerans
Rhodococcus equi
Rothia dentocariosa
Actinomyces israelii
Clavibacter michiganensis
Corynebacterium serosis 354
Corynebacterium amycolatum
Corynebacterium bovis
Corynebacterium jeikeium
Corynebacterium sp.
Corynebacterium pseudodiptheriticum
Corynebacterium diptheriae
Mycobacterium scrofulaceum
Mycobacterium xenopi
Norcardia nova
Norcardia asteroides
Propionibacterium acnes
Tropheryma whipplei
Actinomyces naeslundii
Proteobacteria-γ
Acinetobacter baumannii
Acinetobacter lwoffii
Acinetobacter anitratus
Tatlockia micdadei
Legionella longbeachae
Legionella pneumophila
Coxiella burnetii
Acinetobacter calcoaceticus
Acinetobacter radioresistans
Pseudomonas syringae pv. glycinea
Francisella tularensis
Mollicutes
Mycoplasma mycoides
Erysipelothrix rhusiopathiae
Mycoplasma pneumonia
Pseudomonas aeruginosa 363
Pseudomonas alcaligenes
Pseudomonas fluorescens
Pseudomonas stutzeri
Pseudomonas putida
Proteobacteria-γ 354
Haemophilus ducreyi
Haemophilus influenzae
Haemophilus paraphrophilus
Vibrio cholerae
Haemophilus parainfluenzae
Xanthomonas oryzae pv. oryzicola
Pasteurella multocida
Xylella fastidiosa
Aeromonas hydrophila
Alteromonas tetradonis
Vibrio parahaemolyticus
Haemophilus parahaemolyticus
Stenotrophomonas maltophila
Haemophilus aegyptius
Xanthomonas malvacearum
Haemophilus aphrophilus
Xanthomonas oryzae pv. Oryzae
Vibrio vulnificus
Actinobacillus actinomycetemcomitans
Aeromonas spp.
358, 359
Citrobacter freundii
Escherichia coli
Edwarsiella tarda
Klebsiella pneumoniae
Enterobacter aerogenes
Salmonella enteriditis
Klebsiella oxytoca
Salmoella paratyphi
Moraxella catarrhalis
Salmonella typhi
Morganella morganii
Salmonella typhimurium
Plesiomonas shigelloides
Serratia marcescens
Proteus mirabilis
Shigella dysenteriae
Proteus vulgaris
Shigella flexneri
Providencia sp.
Shigella sonnei
Providencia stuartii
Yersinia enterocolitica
Salmonella choleraesuis
Yersinia pestis
Yersinis pseudotuberculosis
Enterobacter cloacae
Firmicutes
Clostridia
Eubacterium sp.
Clostridium perfringens
Anaerococcus prevotii
Clostridium septicum
Clostridium botulinum
Clostridium tetani
Clostridium difficile
Peptococcus niger
Peptostreptococcus anaerobius
Veillonella dispar
356, 449
Fusobacteria 354
Fusobacterium necrophorum
Streptobacillus moniliformis
Bacilli 362
Streptococcus agalactaie
Streptococcus intermedius
Listeria monocytogenes
Streptococcus bovis
Enterococcus faecium
Staphylococcus intermedius
Streptococcus milleri
Streptococcus pneumoniae
Staphylococcus aureus
Streptococcus equi
Streptococcus pyogenes
Staphylococcus saprophyticus
Enterococcus faecalis
Enterococcus flavescens
Staphylococcus coagulase-neg.
Staphylococcus epidermis
Staphylococcus haemolyticus
Streptococcus viridans
355
350, 351, 353
Bacillus anthracis
Bacillus cereus
Bacillus thuringiensis

Figure 3

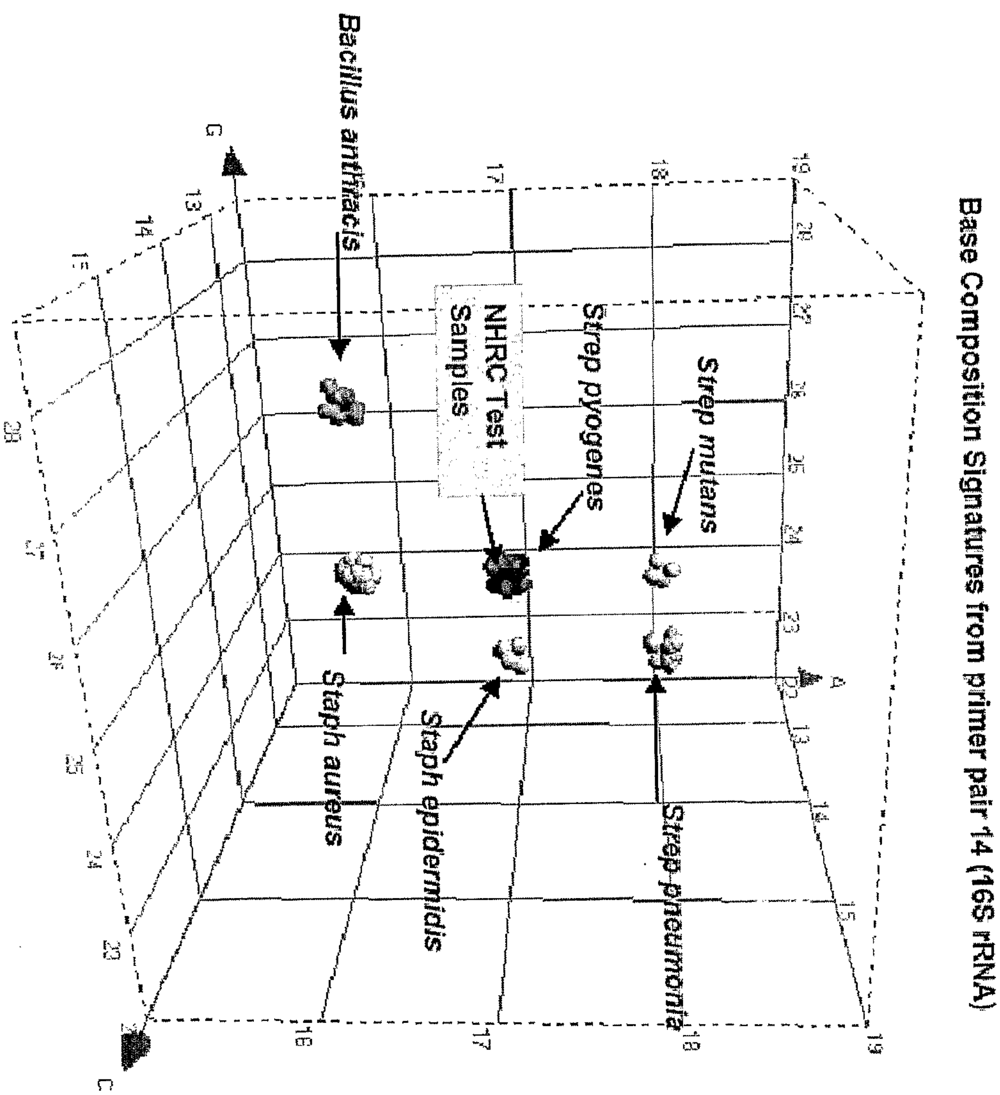

Base Composition Signatures from primer pair 14 (16S rRNA)
Bacillus anthracis
NHRC Test Samples
Strep pyogenes
Strep mutans
Staph aureus
Staph epidermidis
Strep pneumonia
G
A
C

Figure 4

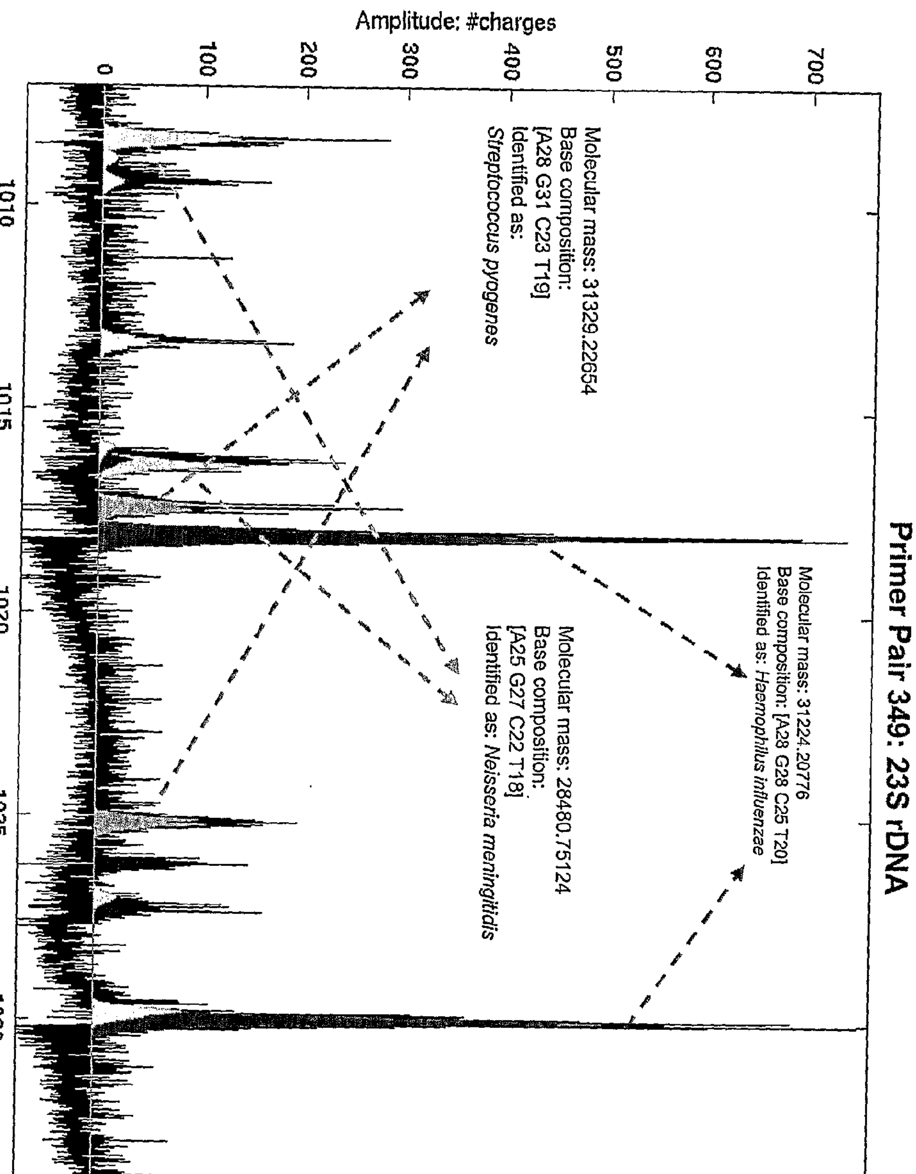
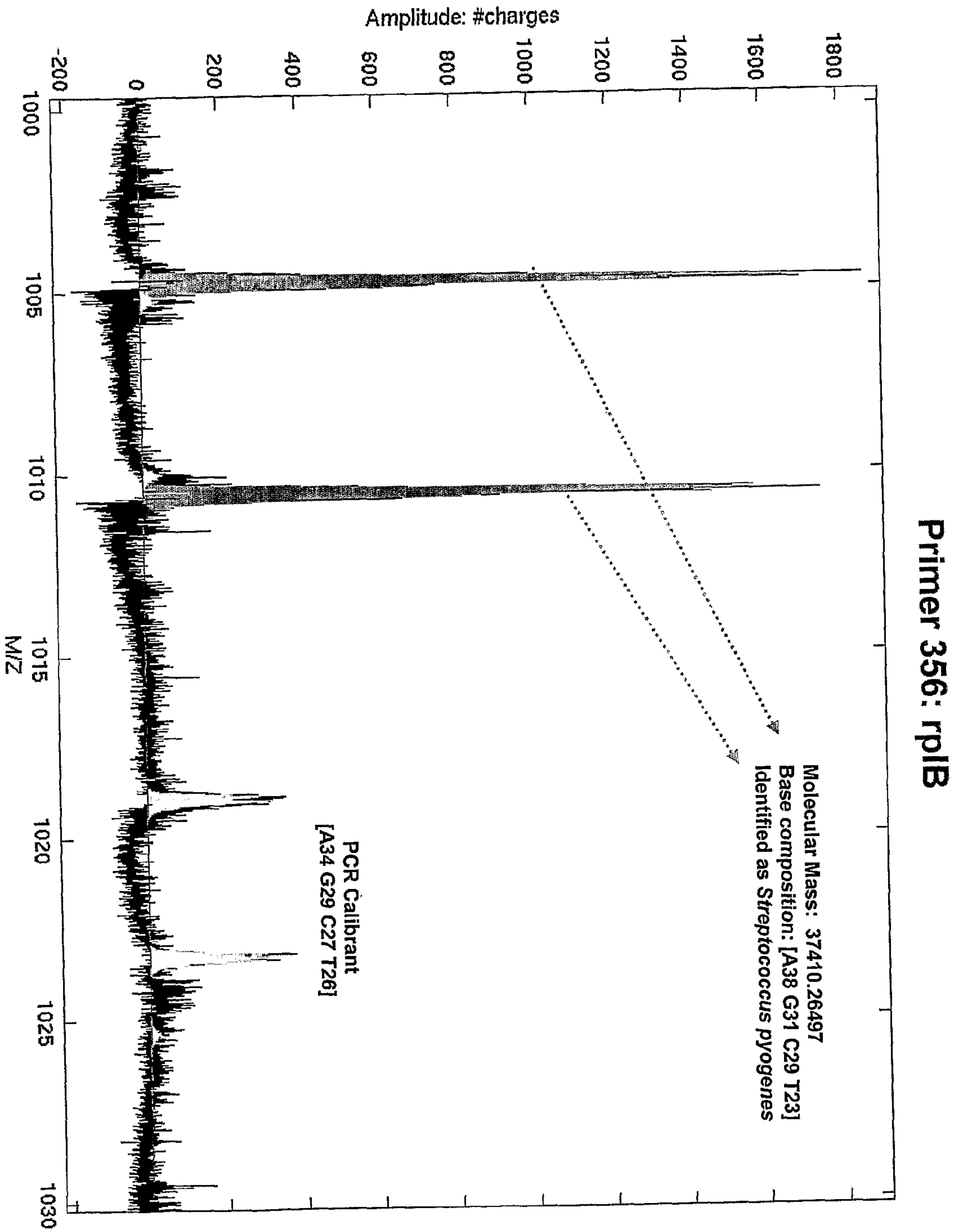

Primer Pair 349: 23S rDNA
Amplitude: #charges
0
100
200
300
400
500
600
700
1010
1015
1020
1025
1030
1035
M/Z
Molecular mass: 31329.22654
Base composition:
[A28 G31 C23 T19]
Identified as:
Streptococcus pyogenes
Molecular mass: 31224.20776
Base composition: [A28 G28 C25 T20]
Identified as: Haemophilus influenzae
Molecular mass: 28480.75124
Base composition:
[A25 G27 C22 T18]
Identified as: Neisseria meningitidis

Figure 5



Primer 356: rplB
Amplitude: #charges
-200
0
200
400
600
800
1000
1200
1400
1600
1800
1000
1005
1010
1015
1020
1025
1030
M/Z
Molecular Mass: 37410.26497
Base composition: [A38 G31 C29 T23]
Identified as *Streptococcus pyogenes*
PCR Calibrant
[A34 G29 C27 T26]

Figure 6

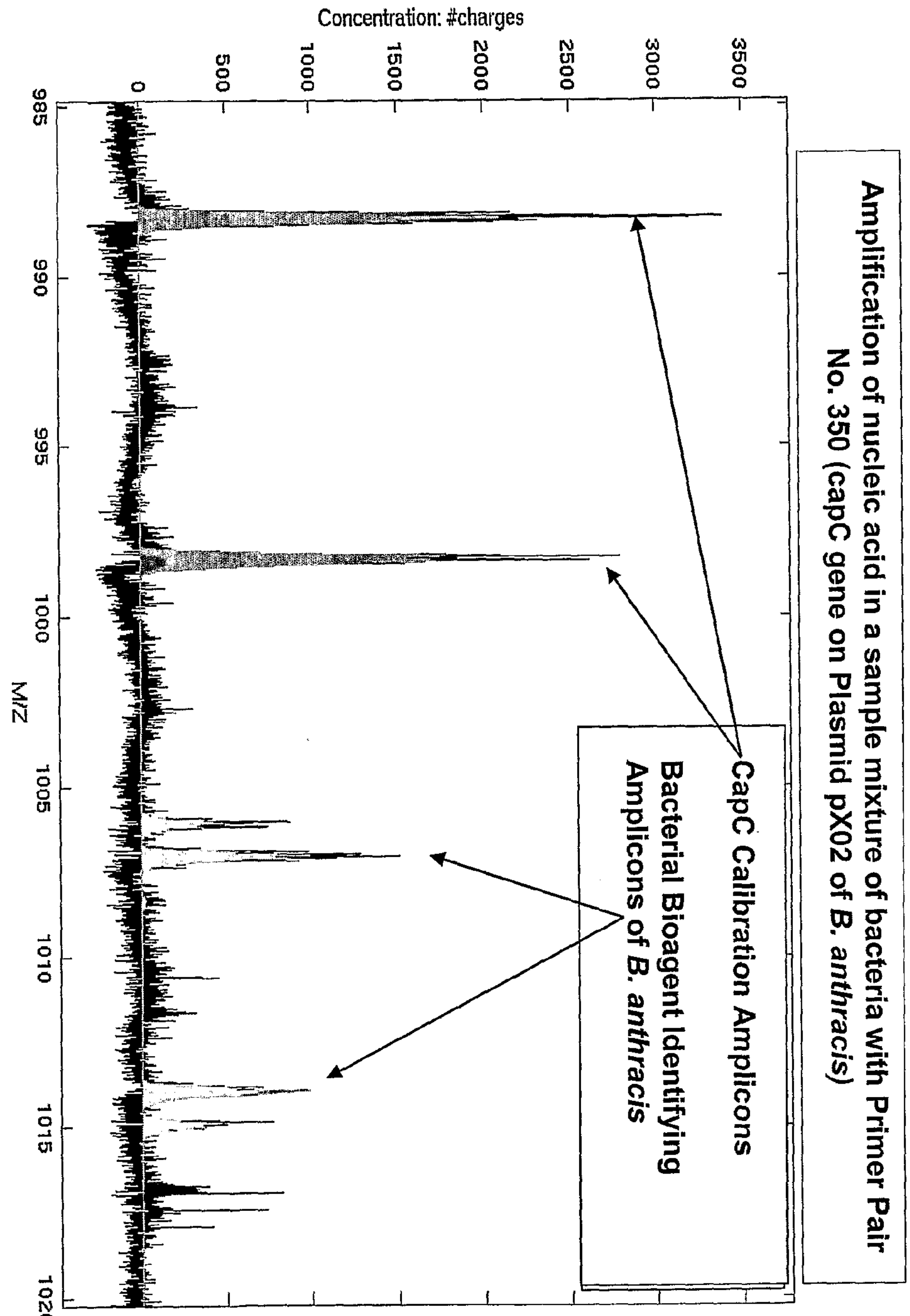

Amplification of nucleic acid in a sample mixture of bacteria with Primer Pair No. 350 (capC gene on Plasmid pX02 of *B. anthracis*)
CapC Calibration Amplicons
Bacterial Bioagent Identifying Amplicons of *B. anthracis*
Concentration: #charges
0
500
1000
1500
2000
2500
3000
3500
985
990
995
1000
1005
1010
1015
1020
M/Z

Figure 7

SEQUENCE LISTING

<110> Sampath, Rangarajan
Hall, Thomas A.
Eshoo, Mark W.

<120> COMPOSITIONS FOR IDENTIFICATION OF
BACTERIA

<130> DIBIS-0083WO

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<151> 2005-08-03

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<160> 1464

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<220>
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<210> 4
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<220>
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<210> 7
<211> 18
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<220>
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<210> 9
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer

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<220>
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